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**WO 01/40309 A2**

(54) Title: ANTI-PROSTATE STEM CELL ANTIGEN (PSCA) ANTIBODY COMPOSITIONS AND METHODS OF USE

(57) Abstract: The invention provides isolated anti-prostate stem cell antigen (PSCA) antibodies that internalize upon binding to PSCA on a mammalian in vivo. The invention also encompasses compositions comprising an anti-PSCA antibody and a carrier. These compositions can be provided in an article of manufacture or a kit. Another aspect of the invention is an isolated nucleic acid encoding an anti-PSCA antibody, as well as an expression vector comprising the isolated nucleic acid. Also provided are cells that produce the anti-PSCA antibodies. The invention encompasses a method of producing the anti-PSCA antibodies. Other aspects of the invention are a method of killing a PSCA-expressing cancer cell, comprising contacting the cancer cell with an anti-PSCA antibody and a method of alleviating or treating a PSCA-expressing cancer in a mammal, comprising administering a therapeutically effective amount of the anti-PSCA antibody to the mammal.

## ANTI-PROSTATE STEM CELL ANTIGEN (PSCA) ANTIBODY COMPOSITIONS AND METHODS OF USE

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FIELD OF THE INVENTION

The present invention relates to anti-PSCA antibody compositions and methods of killing PSCA-expressing cancers cells.

BACKGROUND OF THE INVENTION

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In humans, prostate cancer is one of the most commonly diagnosed malignancies in males and is the second leading cause of cancer related death in men. The American Cancer Society estimates that for the year 2000, 180,400 new cases of prostate cancer will be diagnosed with 31,900 deaths from the disease. In advanced stages, prostate cancer metastasizes to the bone. While advances in early diagnosis and treatment of locally confined tumors have been achieved, prostate cancer is incurable once it has metastasized. Patients with metastatic prostate cancer on hormonal therapy will eventually develop an androgen-refractory (androgen independent) state that will lead to disease progression and death. Currently, prostate-specific antigen (PSA) is the most widely used tumor marker for screening, diagnosis, and monitoring prostate cancer. However, widespread use of PSA as a tool for screening is controversial since PSA fails to discriminate accurately between benign and malignant prostate disease.

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Depending on the stage of the cancer, prostate and bladder cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, chemotherapy, androgen deprivation (e.g., hormonal therapy) in the case of prostate cancer. While surgical or radiation therapy significantly improves survival in patients with early stages of the disease, the therapeutic options are very limited for advanced cases, particularly for tumor recurrences following hormone ablation. The majority of patients who undergo hormone therapy progress to develop androgen-independent disease. Currently, there is no effective treatment for the 20-40% of prostate cancer patients who develop recurrent disease after surgery or radiation therapy, or for those in whom the cancer has metastasized at the time of diagnosis. Chemotherapy has its toxic side effects, especially in elderly patients. Development of new forms of therapy especially for disease refractory to androgen deprivation is an urgent need in the management of prostatic carcinoma.

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The identification of a novel cell surface antigen, prostate stem cell antigen (PSCA) has been described, see, e.g., U.S. Patent 5,856,136 (SCAH2), WO 99/14328 (protein PRO232), WO 98/40403 (PSCA), WO 98/51805 (PS116) and Reiter et al. *Proc. Nat. Acad. Sci.* 95:1735-1740 (1998). PSCA was initially cloned from a cDNA library of a LAPC-4 xenograft from a prostate cancer patient.

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PSCA is a GPI-linked molecule of 123 amino acids that is expressed on the surface of a number of cell types including prostate and bladder tumor cells. The gene is located on the *myc* locus on 8Q24.2 which is a region amplified in 80% of prostate cancers. PSCA shows 30% homology with SCA-2. The protein has a hydrophobic signal sequence at the first 20 amino acids of the N-terminus and a GPI-anchoring sequence at amino acid 100-123 of the C-terminus (Fig. 2 on page 1737 of Reiter et al. (1998)). There are four predicted glycosylation sites. The cell surface protein is shed with a  $t_{1/2}$  of about 10 hours in culture. It has been reported that PSCA is widely over-expressed across all stages of prostate cancer, including high grade

prostatic intraepithelial neoplasia (PIN), and both androgen-dependent and -independent prostate tumors. Antibodies that are able to target PSCA-expressing tumor cells *in vivo* and that can internalize upon binding to the cells, have not been reported.

Antibody-based therapy has proved very effective in the treatment of various cancers. For example, HERCEPTIN® and RITUXAN® (both from Genentech, S. San Francisco), have been used successfully to treat breast cancer and non-Hodgkin's lymphoma, respectively. HERCEPTIN® is a recombinant DNA-derived humanized monoclonal antibody that selectively binds to the extracellular domain of the human epidermal growth factor receptor 2 (HER2) proto-oncogene. HER2 protein overexpression is observed in 25-30% of primary breast cancers. RITUXAN® is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. Both these antibodies are produced in CHO cells.

The present invention provides alternative methods of treating cancer that overcome the limitations of conventional therapeutic methods as well as offer additional advantages that will be apparent from the detailed description below.

#### SUMMARY OF THE INVENTION

The invention provides isolated anti-PSCA antibodies that internalize upon binding to PSCA on a mammalian cell *in vivo*. These antibodies can also target a PSCA-expressing tumor cell *in vivo*. In a specific embodiment, the anti-PSCA antibodies internalize upon binding to PSCA on cancer cells, including prostate cancer, urinary tract cancers (e.g., bladder cancer) and lung cancer. Provided are internalizing anti-PSCA antibodies that are monoclonal antibodies. In specific embodiments, the antibodies are 10E3, 6F8, 8D11, 5F2, 6C3, 6B8 and 10C5, produced by the hybridomas deposited under American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, and PTA-2264. The V region sequences of the 6F8, 8D11, 5F2, 6B8 antibodies are shown in Figures 12 (SEQ ID NOs. 3-9). The chimeric murine-human antibody polypeptides having the amino acid sequence of SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, or SEQ ID NO.13, are provided. The invention provides anti-PSCA antibodies and antibody fusion polypeptides that comprise the amino acid sequences of any one of SEQ ID NO. 1-13.

Also provided are antibodies that compete for binding to the same epitope as the epitope bound by any of the aforementioned monoclonal antibodies. In another embodiment, an isolated anti-PSCA monoclonal antibody that inhibits the growth of PSCA-expressing cancer cells *in vivo*, or is cytotoxic *in vivo*, to such cells and tumors containing such cells, is provided.

The invention also provides anti-PSCA antibodies that are conjugated to a cytotoxic agent or to a growth inhibitory agent. The antibodies are internalizing and/or growth inhibitory antibodies. The cytotoxic agent can be a toxin, antibiotic, radioactive isotope or nucleolytic enzyme. In a preferred embodiment, the toxin is a maytansinoid, more preferably the maytansinoid having the structure shown in FIG. 22.

The anti-PSCA antibodies of the preceding embodiments include intact (full length) antibodies as well as antibody fragments. The anti-PSCA antibodies of the invention include human antibodies and chimeric antibodies as well as antibody fusion polypeptides comprising at least the antibody V region sequences fused to a heterologous polypeptide. In a preferred embodiment, the anti-PSCA antibody of any of the preceding embodiments is a chimeric or human antibody. In a preferred embodiment, the chimeric

antibody is a humanized antibody. The humanized anti-PSCA antibodies include humanized forms of any of the antibodies produced by the hybridomas deposited under American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, and PTA-2265 and the chimeric antibodies include those having amino acid sequences of SEQ ID NO.10-13 as shown in FIG. 13. The antibodies of the invention include those produced in mammalian or bacterial cells.

The invention also encompasses a composition comprising any one of the anti-PSCA antibodies of the above embodiments, and a carrier. In one embodiment, the antibody in the composition is a human antibody or a humanized form of the monoclonal antibody produced by any one of the hybridomas deposited under ATCC accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, and PTA-2264. In one specific embodiment, the antibody in the composition is conjugated to a maytansinoid. In a preferred embodiment, the carrier is a pharmaceutically-acceptable carrier. These compositions can be provided in an article of manufacture or a kit.

Another aspect of the invention is an isolated nucleic acid encoding any one of the anti-PSCA antibodies of the above embodiments, as well as an expression vector comprising the isolated nucleic acid operably linked to an expression regulatory sequence.

Also provided by the invention are cells that produce the above-described anti-PSCA antibodies. In one embodiment, the antibody producing cells are hybridoma cells including the specific hybridoma cells having ATCC accession numbers PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, and PTA-2264. In another embodiment, the cell is a bacterial cell. A host cell comprising the above-described vector is specifically provided.

The invention further encompasses a method of producing the anti-PSCA antibodies of the above embodiments, comprising culturing the cells of the above embodiments and recovering the antibody from the cell culture.

Yet a separate aspect of the invention is a method of killing a PSCA-expressing cancer cell, comprising contacting the cancer cell with an anti-PSCA antibody of any of the above embodiments, thereby killing the cancer cell. Another aspect is a method of alleviating or treating a PSCA-expressing cancer in a mammal, comprising administering a therapeutically effective amount of the anti-PSCA antibodies of the invention to the mammal. In preferred embodiments of the preceding two methods, the cancer is a prostate, bladder or lung cancer, more preferably prostate cancer and especially an androgen independent prostate cancer cell or a metastatic prostate cancer. In a preferred embodiment of these methods, the anti-PSCA antibody is a human or a humanized antibody. In another preferred embodiment, the antibody is conjugated to a cytotoxic agent such as a toxin or a radioactive isotope. Preferably, the toxin is calicheamicin or a maytansinoid such as "DM1" having the structure shown in Fig. 22. The method of alleviating the PSCA-expressing cancer anticipates administration of the anti-PSCA antibody in conjunction with chemotherapy wherein the mammal is also receiving at least one chemotherapeutic agent. In a specific embodiment, the chemotherapeutic agent is a taxane such as paclitaxel (TAXOL®) or docetaxel, or derivatives and analogs thereof.

In a further aspect, the invention provides an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an anti-PSCA antibody of the above



embodiments, and further comprising a package insert indicating that the composition can be used to alleviate or treat a PSCA-expressing cancer and in particular, prostate cancer.

#### BRIEF DESCRIPTION OF THE FIGURES

5 Figure 1 shows the binding to and staining of gD-PSCA transfected CHO cells by Mab by 5F2, as assayed by FACS (see Example 1).

Figure 2 shows the binding to and staining of gD-PSCA transfected CHO cells by Mab by 10C5, as assayed by FACS (see Example 1).

10 Figure 3 shows the binding activity of the 6B8.1D7.2B3 anti-PSCA Mab to various soluble PSCA antigens (see Example 1).

Figure 4 shows the binding curve for antibody ascites no. 2403 (5F2) (see Example 1).

Figure 5 is a graph showing internalization of the different anti-PSCA monoclonal antibodies (6C3; 6F8; 10A1; 8D11; 5F2; 7A6) in the three cell types, as measured within 1 hour, by the intensity of the Cy3 fluorescence (see Example 3).

15 Figure 6 is a graph showing surface labeling of PSCA by the different anti-PSCA monoclonal antibodies (6C3; 6F8; 10A1; 8D11; 5F2; 7A6) on the three cell types, as measured by the intensity of the Cy3 fluorescence (see Example 3).

Figure 7 is a graph comparing the amount of surface versus internalized anti-PSCA monoclonal antibody for each of the antibodies as measured within 1 hour, by the intensity of the Cy3 fluorescence. The amount of surface and internalized antibody is an average of the results from the three different cell lines used in Figures 5 and 6.

Figures 8A, 8B and 8C are micrographs showing the distribution of the fluorescent labeled 8D11 anti-PSCA antibody in PSCA-expressing cell lines HCT 47 gD, MCF7/HER2/PSCA, and PC3 19 gD PSCA respectively, as observed by confocal microscopy (See Example 3).

25 Figures 9A-D show electron micrographs of CHO cells expressing PSCA treated with anti-PSCA antibodies labeled with gold adducts. Figures 9A and 9B show cells incubated with antibody 6C3 for 15 minutes and 1 hour, respectively. Figures 9C and 9D show cells incubated with antibody 10E3 for 15 minutes and 1 hour, respectively.

Figure 10 is a micrograph showing internalization of <sup>125</sup>I-8D11 antibody in HCT116 gDPSCA cells 30 15 minutes after contact with the antibody (see Example 3 below).

Figure 11 shows the levels of internalization of three anti-PSCA antibodies [ascites # 2395 (6F8), #2399 (8D11), #2403(5F2)] at 37°C after 5 hours, in Her2 -expressing MCF7 cells transfected with either gD-PSCA or vector alone (see Example 3 below). Herceptin, is an anti-Her2 antibody.

Figure 12 shows the amino acid sequences of the heavy and light chain variable region domains (V<sub>H</sub> and V<sub>L</sub>) of the antibodies from hybridoma clones 6F8.2F4, Asc# 2395; 8D11.2E9, Asc# 2399; 5F2.4H4.1E3, Asc# 2403; and 6B8.1D7.2B3, Asc#2761.

Figure 13 shows the amino acid sequences of the full length chimeric 2403 IgG including the signal peptide sequences, and the sequences of chimeric 6B8 Fab.

Figure 14 shows a comparison of the relative expression levels of PSCA in various tumor versus 40 normal tissues (see Example 4).

Figure 15 shows the DNA and protein sequences of the Cynomolgus monkey PSCA type 1 (SEQ ID NO: 16 and NO:17, respectively), see Example 6. The "o" at the end of the amino acid sequence stands for the Ochre stop codon.

5 Figure 16 shows the DNA and protein sequences of the Cynomolgus monkey PSCA type II (SEQ ID NO: 18 and NO: 19, respectively), see Example 6. The "o" at the end of the amino acid sequence stands for the Ochre stop codon.

Figure 17 compares the amino acid sequences for human PSCA (SEQ ID NO.1) and Cynomolgus monkey type I PSCA (SEQ ID NO. 17).

10 Figure 18 shows the growth of control PC-3 tumor cells (PSCA-negative) in mice treated with 3 of the anti-PSCA antibodies (6B8, 8D11, and 5F2) as compared to the anti-ragweed negative control antibody (see Example 5).

Figure 19 shows the growth of PC-3.gDPSCA tumors in mice treated with 3 of the anti-PSCA antibodies (6F8, 8D11, and 5F2) as compared to the negative control, anti-ragweed antibody. The antibodies were administered by intraperitoneal injection starting one day prior to inoculation with PSCA-transfected PC-3 cells, followed by biweekly injections following cell inoculation. "RX" under the arrows indicates the time point (day) of injection of the antibodies into the mice (see Example 5).

Figure 20 shows the growth of PC-3.gDPSCA tumors treated with antibodies 8D11, 5F2, 6B8 and control ragweed antibodies, as described in Figure 19 (see Example 5).

20 Figure 21 shows the growth of PC-3.gDPSCA tumors in mice wherein treatment with anti-PSCA antibodies 6F8, 8D11, or 5F2 was initiated after the tumors had been allowed to grow for 1 week in the mice and had reached a mean tumor volume of approximately  $200 \text{ mm}^3$  per group of mice. Antibody administration was biweekly. "RX" under the arrows indicates the time point (day) of intraperitoneal injection of the antibodies into the mice (see Example 5).

Figure 22 shows the structure of the maytansinoid designated "DM1" (see Example 7).

25 Figure 23 illustrates the structure of anti-PSCA antibody-DM1 conjugate (see Example 7).

Figure 24 shows the sensitivity of assay cell lines, PC3.Neo cells and PC3.gD human PSCA clone 4 cells, to non-conjugated DM1 (see Example 8).

Figure 25 shows the cytotoxicity of antibody conjugate 2399-DM1 to PC3.gDhuman PSCA clone 4 cells compared to isotype control 1429-DM1 (see Example 8).

30 Figure 26 shows the cytotoxicity of 2761-DM1 to PC3.gDhuman PSCA clone 4 cells compared to isotype control 1428-DM1 (see Example 8).

Figure 27 shows the lack of toxicity of antibody-DM1 conjugates in antigen-negative PC3.Neo cells (see Example 8).

35 Figure 28 shows the growth of PSCA-expressing, PC3 human prostate tumor cells in a mouse model in which the mice were treated with either naked (non-toxin conjugated) antibody or DM1-conjugated antibody. The anti-PSCA antibodies were 2399 or 6B8 and the anti-ragweed antibody 1428 serves as the control antibody (see Example 9).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**I. Definitions**

Human "PSCA" or "prostate stem cell antigen" as used herein, refers to a single subunit glycoprotein of 123 amino acids that is expressed on the cell surface as a glycosylphosphatidylinositol (GPI)-anchored protein, whose nucleotide and amino acid sequence sequences are as disclosed in e.g., U.S. Patent 5,856,136 (SCAH2; SEQ ID NO:4); WO 99/14328 (PRO232, nucleotide sequence in SEQ ID NO:1, Fig. 1; amino acid sequence in Fig. 2; SEQ ID NO:2); Fig. 2 (amino acid sequence) on page 1737 of Reiter et al. *Proc. Nat. Acad. Sci.* 95:1735-1740 (1998); WO 98/51805 (SEQ ID NOs: 12 and 25); and WO 98/40403 (Figures 1A and 1B). The amino acid sequence of murine PSCA is provided in Fig. 2 on page 1737 of Reiter et al. *Proc. Nat. Acad. Sci.* 95:1735-1740 (1998). The protein has a signal sequence at the amino-terminus and the GPI-anchoring sequence is present at the carboxy terminus. In the human PSCA sequence, amino acids 1-20 represent the N-terminal signal sequence which gets cleaved in the mature protein, and amino acid positions 100-123 represent C-terminal GPI-anchoring sequences (Reiter et al., 1998, see Fig.2). Thus, the amino acids 21 to about 100 are presumably on the cell surface. PSCA as used herein include allelic variants and conservative substitution mutants of the protein which have PSCA biological activity. These allelic variants and conservative substitution mutants may be GPI-linked or secreted forms of the protein.

The term "antibody" (Ab) as used herein includes monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity. The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

An "isolated antibody" is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains (an IgM antibody consists of 5 of the basic heterotetramer unit along with an additional polypeptide called J chain, and therefore contain 10 antigen binding sites, while secreted IgA antibodies can polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain units along with J chain). In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to a H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain ( $V_H$ ) followed by three constant domains ( $C_H$ ) for each of the  $\alpha$  and  $\gamma$  chains and four  $C_H$  domains for  $\mu$  and  $\epsilon$  isotypes. Each

L chain has at the N-terminus, a variable domain ( $V_L$ ) followed by a constant domain ( $C_L$ ) at its other end. The  $V_L$  is aligned with the  $V_H$  and the  $C_L$  is aligned with the first constant domain of the heavy chain ( $C_{H1}$ ). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a  $V_H$  and  $V_L$  together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains ( $C_H$ ), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$  and  $\mu$ , respectively. The  $\gamma$  and  $\alpha$  classes are further divided into subclasses on the basis of relatively minor differences in  $C_H$  sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and define specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a  $\beta$ -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the  $V_L$ , and around about 1-35 (H1), 50-65 (H2) and 95-102 (H3) in the  $V_H$ ; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the  $V_L$ , and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the  $V_H$ ; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal

antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present invention may be prepared by the hybridoma methodology first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example.

The monoclonal antibodies herein include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see U.S. Patent No. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (*e.g.* Old World Monkey, Ape etc), and human constant region sequences.

An "intact" antibody is one which comprises an antigen-binding site as well as a C<sub>L</sub> and at least heavy chain constant domains, C<sub>H</sub>1, C<sub>H</sub>2 and C<sub>H</sub>3. The constant domains may be native sequence constant domains (*e.g.* human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

An "antibody fragment" comprises a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies (see US patent 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V<sub>H</sub>), and the first constant domain of one heavy chain (C<sub>H</sub>1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')<sub>2</sub> fragment which roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having additional few residues at the carboxy terminus of the C<sub>H</sub>1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')<sub>2</sub> antibody fragments originally were produced as pairs of

Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

5 The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, which region is also the part recognized by Fc receptors (FcR) found on certain types of cells.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen  
10 binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

"Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the V<sub>H</sub> and V<sub>L</sub> antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further  
15 comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); Borrebaeck 1995, infra.

The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments  
20 (see preceding paragraph) with short linkers (about 5-10 residues) between the V<sub>H</sub> and V<sub>L</sub> domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, i.e., fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V<sub>H</sub> and V<sub>L</sub> domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., Proc.  
25 Natl. Acad. Sci. USA, 90:6444-6448 (1993).

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (e.g., antibody) derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of a naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other  
30 mammalian species.

The term "amino acid sequence variant" refers to a polypeptide that has amino acid sequences that differ to some extent from a native sequence polypeptide. Ordinarily, amino acid sequence variants of PSCA will possess at least about 70% homology with the native sequence PSCA, preferably, at least about 80%, more preferably at least about 85%, even more preferably at least about 90% homology, and most preferably  
35 at least 95%. The amino acid sequence variants can possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence.

The phrase "functional fragment or analog" of an antibody is a compound having qualitative biological activity in common with a full-length antibody. For example, a functional fragment or analog of an anti-IgE antibody is one which can bind to an IgE immunoglobulin in such a manner so as to prevent or

substantially reduce the ability of such molecule from having the ability to bind to the high affinity receptor, FcεRI.

“Homology” is defined as the percentage of residues in the amino acid sequence variant that are identical after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent  
5 homology. Methods and computer programs for the alignment are well known in the art. One such computer program is "Align 2", authored by Genentech, Inc., which was filed with user documentation in the United States Copyright Office, Washington, DC 20559, on December 10, 1991.

"Humanized" forms of non-human (e.g., rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the most part, humanized antibodies are  
10 human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity, and capability. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not  
15 found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an  
20 immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

As used herein, an anti-PSCA antibody that "internalizes" is one that is taken up by (i.e., enters) the cell upon binding to PSCA on a mammalian cell (i.e. cell surface PSCA). The internalizing antibody will of  
25 course include antibody fragments, human or humanized antibody and antibody conjugate. For therapeutic applications, internalization in vivo is contemplated. The number of antibody molecules internalized will be sufficient or adequate to kill a PSCA-expressing cell, especially a PSCA-expressing cancer cell. Depending on the potency of the antibody or antibody conjugate, in some instances, the uptake of a single antibody molecule into the cell is sufficient to kill the target cell to which the antibody binds. For example, certain  
30 toxins are highly potent in killing such that internalization of one molecule of the toxin conjugate to the antibody is sufficient to kill the tumor cell.

Whether an anti-PSCA antibody internalizes upon binding PSCA on a mammalian cell can be determined by various assays including those described in the experimental examples below. For example, to test internalization in vivo, the test antibody is labeled and introduced into an animal known to have PSCA  
35 expressed on the surface of certain cells. The antibody can be radiolabeled or labeled with fluorescent or gold particles, for instance. Animals suitable for this assay include a mammal such as a NCR nude mouse that contains a human PSCA-expressing tumor transplant or xenograft, or a mouse into which cells transfected with human PSCA have been introduced, or a transgenic mouse expressing the human PSCA transgene. Appropriate controls include animals that did not receive the test antibody or that received an  
40 unrelated antibody, and animals that received an antibody to another antigen on the cells of interest, which antibody is known to be internalized upon binding to the antigen (e.g., HERCEPTIN which binds to Her2

expressed on the human breast tumor cell line, MCF-7 in Example 3). The antibody can be administered to the animal, e.g., by intravenous injection. At suitable time intervals, tissue sections of the animal can be prepared using known methods or as described in the experimental examples below, and analyzed by light microscopy or electron microscopy, for internalization as well as the location of the internalized antibody in the cell. For internalization *in vitro*, the cells can be incubated in tissue culture dishes in the presence or absence of the relevant antibodies added to the culture media and processed for microscopic analysis at desired time points. The presence of an internalized, labeled antibody in the cells can be directly visualized by microscopy or by autoradiography if radiolabeled antibody is used. Alternatively, in a quantitative biochemical assay, a population of cells comprising PSCA-expressing cells are contacted *in vitro* or *in vivo* with a radiolabeled test antibody and the cells (if contacted *in vivo*, cells are then isolated after a suitable amount of time) are treated with a protease or subjected to an acid wash to remove uninternalized antibody on the cell surface. The cells are ground up and the amount of protease resistant, radioactive counts per minute (cpm) associated with each batch of cells is measured by passing the homogenate through a scintillation counter. Based on the known specific activity of the radiolabeled antibody, the number of antibody molecules internalized per cell can be deduced from the scintillation counts of the ground-up cells. Cells are "contacted" with antibody *in vitro* preferably in solution form such as by adding the cells to the cell culture media in the culture dish or flask and mixing the antibody well with the media to ensure uniform exposure of the cells to the antibody. Instead of adding to the culture media, the cells can be contacted with the test antibody in an isotonic solution such as PBS in a test tube for the desired time period. *In vivo*, the cells are contacted with antibody by any suitable method of administering the test antibody such as the methods of administration described below when administered to a patient.

The faster the rate of internalization of the antibody upon binding to the PSCA expressing cell *in vivo*, the faster the desired killing or growth inhibitory effect on the target PSCA-expressing cell can be achieved, e.g., by a cytotoxic immunoconjugate. Preferably, the kinetics of internalization of the anti-PSCA antibodies are such that they favor rapid killing of the PSCA-expressing target cell. Therefore, it is desirable that the anti-PSCA antibody exhibit a rapid rate of internalization preferably, within 24 hours from administration of the antibody *in vivo*, more preferably within about 12 hours, even more preferably within about 30 minutes to 1 hour, and most preferably, within about 30 minutes. The present invention provides antibodies that internalize as fast as about 15 minutes from the time of introducing the anti-PSCA antibody *in vivo*. The antibody will preferably be internalized into the cell within a few hours upon binding to PSCA on the cell surface, preferably within 1 hour, even more preferably within 15-30 minutes.

To determine if a test antibody can compete for binding to the same epitope as the epitope bound by the anti-PSCA antibodies of the present invention including the antibodies produced by the hybridomas deposited with the ATCC, a cross-blocking assay e.g., a competitive ELISA assay can be performed. In an exemplary competitive ELISA assay, PSCA coated on the wells of a microtiter plate is pre-incubated with or without candidate competing antibody and then the biotin-labeled anti-PSCA antibody of the invention is added. The amount of labeled anti-PSCA antibody bound to the PSCA antigen in the wells is measured using avidin-peroxidase conjugate and appropriate substrate. The antibody can be labeled with a radioactive or fluorescent label or some other detectable and measurable label. The amount of labeled anti-PSCA antibody that bound to the antigen will have an indirect correlation to the ability of the candidate competing antibody (test antibody) to compete for binding to the same epitope, i.e., the greater the affinity of the test antibody for the same epitope, the



less labeled antibody will be bound to the antigen-coated wells. A candidate competing antibody is considered an antibody that binds substantially to the same epitope or that competes for binding to the same epitope as an anti-PSCA antibody of the invention if the candidate antibody can block binding of the PSCA antibody by at least 20%, preferably by at least 20-50%, even more preferably, by at least 50% as compared to the control performed in parallel in the absence of the candidate competing antibody (but may be in the presence of a known non-competing antibody). It will be understood that variations of this assay can be performed to arrive at the same quantitative value.

An antibody having a "biological characteristic" of a designated antibody, such as any of the monoclonal antibodies 10E3, 6F8, 8D11 and 5F2, is one which possesses one or more of the biological characteristics of that antibody which distinguish it from other antibodies that bind to the same antigen, PSCA. For example, an antibody with a biological characteristic of 6F8 will bind the same epitope as that bound by 6F8 (*e.g.* which competes for binding or blocks binding of monoclonal antibody 6F8 to PSCA), be able to target a PSCA expressing tumor cell *in vivo* and will internalize upon binding to PSCA on a mammalian cell *in vivo*. Likewise, an antibody with the biological characteristic of the 5F2 or 6B8 antibody will have the same epitope binding, targeting, internalizing, tumor growth inhibitory and cytotoxic properties of the antibody.

The term "antagonist" antibody is used in the broadest sense, and includes an antibody that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PSCA protein disclosed herein. Methods for identifying antagonists of a PSCA polypeptide may comprise contacting a PSCA polypeptide or a cell expressing PSCA on the cell surface, with a candidate antagonist antibody and measuring a detectable change in one or more biological activities normally associated with the PSCA polypeptide.

An "antibody that inhibits the growth of tumor cells expressing PSCA" or a "growth inhibitory" antibody is one which binds to and results in measurable growth inhibition of cancer cells expressing or overexpressing PSCA. Preferred growth inhibitory anti-PSCA antibodies inhibit growth of PSCA-expressing tumor cells (*e.g.*, prostate cancer cells) by greater than 20%, preferably from about 20% to about 50%, and even more preferably, by greater than 50% (*e.g.* from about 50% to about 100%) as compared to the appropriate control, the control typically being tumor cells not treated with the antibody being tested. Growth inhibition can be measured at an antibody concentration of about 0.1 to 30  $\mu\text{g/ml}$  or about 0.5 nM to 200 nM in cell culture, where the growth inhibition is determined 1-10 days after exposure of the tumor cells to the antibody. Growth inhibition of tumor cells *in vivo* can be determined in various ways such as is described in the Experimental Examples section below. The antibody is growth inhibitory *in vivo* if administration of the anti-PSCA antibody at about 1  $\mu\text{g/kg}$  to about 100  $\text{mg/kg}$  body weight results in reduction in tumor size or tumor cell proliferation within about 5 days to 3 months from the first administration of the antibody, preferably within about 5 to 30 days.

An antibody which "induces apoptosis" is one which induces programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is usually one which overexpresses PSCA. Preferably the cell is a tumor cell, *e.g.* a prostate, breast, ovarian, stomach, endometrial, lung, kidney, colon, bladder cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by

annexin binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an annexin binding assay.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor); and B cell activation.

"Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.* Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express Fc $\gamma$ RIII only, whereas monocytes express Fc $\gamma$ RI, Fc $\gamma$ RII and Fc $\gamma$ RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998).

"Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the Fc $\gamma$ RI, Fc $\gamma$ RII, and Fc $\gamma$ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. Fc $\gamma$ RII receptors include Fc $\gamma$ RIIA (an "activating receptor") and Fc $\gamma$ RIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor Fc $\gamma$ RIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor Fc $\gamma$ RIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see review M. in Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *J. Immunol.* 24:249 (1994)).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least Fc $\gamma$ RIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer

(NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source, *e.g.* from blood.

“Complement dependent cytotoxicity” or “CDC” refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.* as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996), may be performed.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (*e.g.* epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain, as well as head and neck cancer, and associated metastases.

A “PSCA-expressing cell” is a cell which expresses endogenous or transfected PSCA on the cell surface. A “PSCA-expressing cancer” is a cancer comprising cells that have PSCA protein present on the cell surface. A “PSCA-expressing cancer” produces sufficient levels of PSCA on the surface of cells thereof, such that an anti-PSCA antibody can bind thereto and have a therapeutic effect with respect to the cancer. A cancer which “overexpresses” PSCA is one which has significantly higher levels of PSCA at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. PSCA overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the PSCA protein present on the surface of a cell (*e.g.* via an immunohistochemistry assay; FACS analysis). Alternatively, or additionally, one may measure levels of PSCA-encoding nucleic acid or mRNA in the cell, *e.g.* via fluorescent *in situ* hybridization; (FISH; see WO98/45479 published October, 1998), Southern blotting, Northern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study PSCA overexpression by measuring shed antigen in a biological fluid such as serum, *e.g.* using antibody-based assays (see also, *e.g.*, U.S. Patent No. 4,933,294 issued June 12, 1990; WO91/05264 published April 18, 1991; U.S. Patent 5,401,638 issued March 28, 1995; and Sias *et al.* *J. Immunol. Methods* 132: 73-80 (1990)). Aside from the above assays, various *in vivo* assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.* a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.* by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody. A PSCA-expressing cancer includes prostate, bladder, lung, uterine and breast cancer.

A “mammal” for purposes of treating a cancer or alleviating the symptoms of cancer, refers to any mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

"Treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. A subject or mammal is successfully "treated" for a PSCA-expressing cancer if, after receiving a therapeutic amount of an anti-PSCA antibody according to the methods of the present invention, the patient shows observable and/or measurable reduction in or absence of one or more of the following: reduction of PSA levels, reduction in the number of cancer cells or absence of the cancer cells; reduction in the tumor size; inhibition (*i.e.*, slow to some extent and preferably stop) of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition (*i.e.*, slow to some extent and preferably stop) of tumor metastasis; inhibition, to some extent, of tumor growth; and/or relief to some extent, one or more of the symptoms associated with the specific cancer; reduced morbidity and mortality, and improvement in quality of life issues. To the extent the anti-PSCA antibody may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. Reduction of these signs or symptoms may also be felt by the patient.

The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician. For cancer therapy, efficacy can be measured, for example, by assessing the time to disease progression (TTP) and/or determining the response rate (RR). For prostate cancer, the progress of therapy can be assessed by routine methods, usually by measuring serum PSA (prostate specific antigen) levels; the higher the level of PSA in the blood, the more extensive the cancer. Commercial assays for detecting PSA are available, e.g. Hybitech Tandem-E and Tandem-R PSA assay kits, the Yang ProsCheck polyclonal assay (Yang Labs, Bellevue, WA), Abbott Imx (Abbott Labs, Abbott Park, IL), etc. Metastasis can be determined by staging tests and by bone scan and tests for calcium level and other enzymes to determine spread to the bone. CT scans can also be done to look for spread to the pelvis and lymph nodes in the area. Chest X-rays and measurement of liver enzyme levels by known methods are used to look for metastasis to the lungs and liver, respectively. Other routine methods for monitoring the disease include transrectal ultrasonography (TRUS) and transrectal needle biopsy (TRNB).

For bladder cancer, which is a more localized cancer, methods to determine progress of disease include urinary cytologic evaluation by cystoscopy, monitoring for presence of blood in the urine, visualization of the urothelial tract by sonography or an intravenous pyelogram, computed tomography (CT) and magnetic resonance imaging (MRI). The presence of distant metastases can be assessed by CT of the abdomen, chest x-rays, or radionuclide imaging of the skeleton.

The term "therapeutically effective amount" refers to an amount of an antibody or a drug effective to "treat" a disease or disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. See preceding definition of "treating". To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time.

"Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

5 "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum  
10 albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN<sup>TM</sup>, polyethylene glycol (PEG), and PLURONIC<sup>TM</sup>.

15 The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At<sup>211</sup>, I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup>, Re<sup>186</sup>, Re<sup>188</sup>, Sm<sup>153</sup>, Bi<sup>212</sup>, P<sup>32</sup> and radioactive isotopes of Lu), chemotherapeutic agents e.g. methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such  
20 as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits  
25 growth of a cell, especially a PSCA expressing cancer cell, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of PSCA expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin,  
30 daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc  
35 Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

"Label" as used herein refers to a detectable compound or composition which is conjugated directly  
40 or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g.

radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

The term "epitope tagged" used herein refers to a chimeric polypeptide comprising an anti-PSCA antibody polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the Ig polypeptide to which it is fused. The tag polypeptide is also preferably fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

10 A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

15 An "isolated nucleic acid" is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

20 "Vector" includes shuttle and expression vectors. Typically, the plasmid construct will also include an origin of replication (e.g., the ColE1 origin of replication) and a selectable marker (e.g., ampicillin or tetracycline resistance), for replication and selection, respectively, of the plasmids in bacteria. An "expression vector" refers to a vector that contains the necessary control sequences or regulatory elements for expression of the antibodies including antibody fragment of the invention, in bacterial or eukaryotic cells. 25 Suitable vectors are disclosed below.

The cell that produces an anti-PSCA antibody of the invention will include the parent hybridoma cell e.g., the hybridomas that are deposited with the ATCC, as well as bacterial and eukaryotic host cells into which nucleic acid encoding the antibodies have been introduced. Suitable host cells are disclosed below.

## 30 II. Compositions and Methods of the Invention

The invention provides anti-PSCA antibodies. In a preferred embodiment, the anti-PSCA antibodies internalize upon binding to cell surface PSCA on a mammalian cell. In another preferred embodiment, the anti-PSCA antibodies destroy or lead to the destruction of tumor cells bearing PSCA.

35 The pathway and kinetics of internalization of GPI anchored molecules has not been as well studied as the clathrin coated receptor-mediated internalization pathway. It was not apparent that PSCA was internalization-competent. In addition, the ability of an antibody to internalize depends on several factors including the affinity, avidity, and isotype of the antibody, and the epitope that it binds. We have demonstrated herein that the GPI-linked, cell surface PSCA is internalization competent upon binding by the anti-PSCA antibodies of the invention. Additionally, it was demonstrated that the anti-PSCA antibodies of 40 the present invention can specifically target PSCA-expressing tumor cells in vivo and inhibit or kill these cells. These in vivo tumor targeting, internalization and growth inhibitory properties of the anti-PSCA

antibodies make these antibodies very suitable for therapeutic uses, e.g., in the treatment of various cancers including prostate, urinary tract and lung cancer. Internalization of the anti-PSCA antibody is preferred, e.g., if the antibody or antibody conjugate has an intracellular site of action and if the cytotoxic agent conjugated to the antibody does not readily cross the plasma (e.g., the toxin, calicheamicin). Internalization is not  
5 necessary if the antibodies or the agent conjugated to the antibodies do not have intracellular sites of action, e.g., if the antibody can kill the tumor cell by ADCC or some other mechanism.

The anti-PSCA antibodies of the invention also have various non-therapeutic applications. In view of the fact that the use of PSA as a tool for screening or diagnosing prostate cancer is controversial, the anti-PSCA antibodies of the present invention can be useful for diagnosis and staging of PSCA-expressing  
10 cancers (e.g., in radioimaging). The antibodies are also useful for purification or immunoprecipitation of PSCA from cells, for detection and quantitation of PSCA in vitro, e.g. in an ELISA or a Western blot, to kill and eliminate PSCA-expressing cells from a population of mixed cells as a step in the purification of other cells.

The internalizing anti-PSCA antibodies of the invention can be in the different forms encompassed  
15 by the definition of "antibody" herein. Thus, the antibodies include full length or intact antibody, antibody fragments, native sequence antibody or amino acid variants, humanized, chimeric or fusion antibodies, immunoconjugates, and functional fragments thereof. In fusion antibodies an antibody sequence is fused to a heterologous polypeptide sequence. The antibodies can be modified in the Fc region to provide desired effector functions. As discussed in more detail in the sections below, with the appropriate Fc regions, the  
20 naked antibody bound on the cell surface can induce cytotoxicity, e.g., via antibody-dependent cellular cytotoxicity (ADCC) or by recruiting complement in complement dependent cytotoxicity, or some other mechanism. Alternatively, where it is desirable to eliminate or reduce effector function, so as to minimize side effects or therapeutic complications, certain other Fc regions may be used.

In one embodiment, the antibody competes for binding or bind substantially to, the same epitope as  
25 the antibodies of the invention. Antibodies having the biological characteristics of the present anti-PSCA antibodies of the invention are also contemplated, e.g., an anti-PSCA antibody which has the biological characteristics of a monoclonal antibody produced by the hybridomas accorded ATCC accession numbers PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, or PTA-2264, specifically including the in vivo tumor targeting, internalization and any cell proliferation inhibition or cytotoxic characteristics.

30 Specifically provided are anti-PSCA antibodies that bind to an epitope present in amino acids 21-40, or 41-60, or 61-80, or 81-100 of human PSCA, shown in SEQ ID NO.1.

Methods of producing the above antibodies are described in detail below.

The present anti-PSCA antibodies are useful for treating a PSCA-expressing cancer or alleviating one or more symptoms of the cancer in a mammal. Such a cancer includes prostate cancer, cancer of the  
35 urinary tract, lung cancer, breast cancer, colon cancer and ovarian cancer, more specifically, prostate adenocarcinoma, renal cell carcinomas, colorectal adenocarcinomas, lung adenocarcinomas, lung squamous cell carcinomas, and pleural mesothelioma. The cancers encompass metastatic cancers of any of the preceding, e.g., prostate cancer metastases. The antibody is able to bind to at least a portion of the cancer cells that express PSCA in the mammal and preferably is one that does not induce or that minimizes HAMA  
40 response. In a preferred embodiment, the antibody is effective to destroy or kill PSCA-expressing tumor cells or inhibit the growth of such tumor cells, in vitro or in vivo, upon binding to PSCA on the cell. Such an

antibody includes a naked anti-PSCA antibody (not conjugated to any agent). Naked anti-PSCA antibodies having tumor growth inhibition properties *in vivo* include the antibodies described in the Experimental Examples below. Naked antibodies that have cytotoxic or cell growth inhibition properties can be further harnessed with a cytotoxic agent to render them even more potent in tumor cell destruction. Cytotoxic properties can be conferred to an anti-PSCA antibody by, e.g., conjugating the antibody with a cytotoxic agent, to form an immunoconjugate as described below. The cytotoxic agent or a growth inhibitory agent is preferably a small molecule. Toxins such as calicheamicin or a maytansinoid and analogs or derivatives thereof, are preferable.

The invention provides a composition comprising an anti-PSCA antibody of the invention, and a carrier. For the purposes of treating cancer, compositions can be administered to the patient in need of such treatment, wherein the composition can comprise one or more anti-PSCA antibodies present as an immunoconjugate or as the naked antibody. In a further embodiment, the compositions can comprise these antibodies in combination with other therapeutic agents such as cytotoxic or growth inhibitory agents, including chemotherapeutic agents. The invention also provides formulations comprising an anti-PSCA antibody of the invention, and a carrier. In one embodiment, the formulation is a therapeutic formulation comprising a pharmaceutically acceptable carrier.

Another aspect of the invention is isolated nucleic acids encoding the internalizing anti-PSCA antibodies. Nucleic acids encoding both the H and L chains and especially the hypervariable region residues, chains which encode the native sequence antibody as well as variants, modifications and humanized versions of the antibody, are encompassed.

The invention also provides methods useful for treating a PSCA-expressing cancer or alleviating one or more symptoms of the cancer in a mammal, comprising administering a therapeutically effective amount of an internalizing anti-PSCA antibody to the mammal. The antibody therapeutic compositions can be administered short term (acute) or chronic, or intermittent as directed by physician. Also provided are methods of inhibiting the growth of, and killing a PSCA expressing cell.

Finally, the invention also provides kits and articles of manufacture comprising at least one internalizing anti-PSCA antibody. Kits containing anti-PSCA antibodies find use e.g., for PSCA cell killing assays, for purification or immunoprecipitation of PSCA from cells. For example, for isolation and purification of PSCA, the kit can contain an anti-PSCA antibody coupled to beads (e.g., sepharose beads). Kits can be provided which contain the antibodies for detection and quantitation of PSCA *in vitro*, e.g. in an ELISA or a Western blot. Such antibody useful for detection may be provided with a label such as a fluorescent or radiolabel.

### III. Production of anti-PSCA Antibodies

The following describes exemplary techniques for the production of the antibodies useful in the present invention. Some of these techniques are described further in Example 1 and Table 2A. The PSCA antigen to be used for production of antibodies may be, e.g., the full length polypeptide or a portion thereof, including a soluble form of PSCA lacking the GPI anchor sequence (which can be obtained by enzymatic cleavage with phosphatidylinositol phospholipases), or synthetic peptides to selected portions of the protein. Alternatively, cells expressing PSCA at their cell surface (e.g. CHO or NIH-3T3 cells transformed to overexpress PSCA; prostate or other PSCA-expressing tumor cell line), or membranes prepared from such



cells can be used to generate antibodies. The nucleotide and amino acid sequences of human and murine PSCA are available as provided above. PSCA can be produced recombinantly in and isolated from, bacterial or eukaryotic cells using standard recombinant DNA methodology. PSCA can be expressed as a tagged (e.g., epitope tag) or other fusion protein to facilitate isolation as well as identification in various assays.

- 5 Antibodies or binding proteins that bind to various tags and fusion sequences are available as elaborated below. Other forms of PSCA useful for generating antibodies will be apparent to those skilled in the art.

#### Tags

- Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., *Mol. Cell. Biol.*, 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., *Protein Engineering*, 3(6):547-553 (1990)]. The FLAG-peptide [Hopp et al., *BioTechnology*, 6:1204-1210 (1988)] is recognized by an anti-FLAG M2 monoclonal antibody (Eastman Kodak Co., New Haven, CT). Purification of a protein containing the FLAG peptide can be performed by immunoaffinity chromatography using an affinity matrix comprising the anti-FLAG M2 monoclonal antibody covalently attached to agarose (Eastman Kodak Co., New Haven, CT). Other tag polypeptides include the KT3 epitope peptide [Martin et al., *Science*, 255:192-194 (1992)]; an  $\alpha$ -tubulin epitope peptide [Skinner et al., *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

#### (i) Polyclonal antibodies

- Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen (especially when synthetic peptides are used) to a protein that is immunogenic in the species to be immunized. For example, the antigen can be conjugated to keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, e.g., maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride,  $\text{SOCl}_2$ , or  $\text{R}^1\text{N}=\text{C}=\text{NR}$ , where R and  $\text{R}^1$  are different alkyl groups.

- Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100  $\mu\text{g}$  or 5  $\mu\text{g}$  of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) *Monoclonal antibodies*

Monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium which medium preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells (also referred to as fusion partner). For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred fusion partner myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 and derivatives e.g., X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); and Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal e.g. by i.p. injection of the cells into mice.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (e.g., using protein A or protein G-Sepharose) or ion-exchange chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, etc.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA that encodes the antibody may be modified to produce chimeric or fusion antibody polypeptides, for example, by substituting human heavy chain and light chain constant domain (C<sub>H</sub> and C<sub>L</sub>) sequences for the homologous murine sequences (U.S. Patent No. 4,816,567; and Morrison, *et al.*, *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by fusing the immunoglobulin coding sequence with all or part of the coding sequence for a non-immunoglobulin polypeptide (heterologous polypeptide). The non-immunoglobulin polypeptide sequences can substitute for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

### (iii) Humanized antibodies

Methods for humanizing non-human antibodies have been described in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature*, 321:522-525 (1986); Reichmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity and HAMA response (human anti-mouse antibody) when the antibody is intended for human therapeutic use. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human V domain sequence which is closest to that of the rodent is identified and the human framework region (FR) within it accepted for the humanized antibody (Sims *et al.*, *J. Immunol.*, 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al.*, *J. Immunol.*, 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high binding affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Various forms of a humanized anti-PSCA antibody are contemplated. For example, the humanized antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody may be an intact antibody, such as an intact IgG1 antibody.

#### (iv) Human antibodies

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J<sub>H</sub>) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggemann *et al.*, *Year in Immuno.*, 7:33 (1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669 (all of GenPharm); 5,545,807; and WO 97/17852.

Alternatively, phage display technology (McCafferty *et al.*, *Nature* 348:552-553 [1990]) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain

gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats, reviewed in, *e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). See, also, U.S. Patent Nos. 5,565,332 and 5,573,905.

As discussed above, human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275).

(v) *Antibody fragments*

In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan *et al.*, *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')<sub>2</sub> fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')<sub>2</sub> fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')<sub>2</sub> fragment with increased *in vivo* half-life comprising a salvage receptor binding epitope residues are described in U.S. Patent No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Patent No. 5,571,894; and U.S. Patent No. 5,587,458. Fv and sFv are the only species with intact combining sites that are devoid of constant regions; thus, they are suitable for reduced nonspecific binding during *in vivo* use. sFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an sFv. See Antibody Engineering, ed. Borrebaeck, *supra*. The antibody fragment may also be a "linear antibody", *e.g.*, as described in U.S. Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) *Bispecific antibodies*

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the PSCA protein. Other such antibodies may combine an PSCA binding site with a binding site for another protein. Alternatively, an anti-PSCA arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16), so as to focus and localize cellular defense mechanisms to the PSCA-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express PSCA. These antibodies possess a PSCA-binding arm and an arm which binds the cytotoxic agent (*e.g.* saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* F(ab)<sub>2</sub> bispecific antibodies).

WO 96/16673 describes a bispecific anti-ErbB2/anti-FcγRIII antibody and U.S. Patent No. 5,837,234 discloses a bispecific anti-ErbB2/anti-FcγRI antibody. A bispecific anti-ErbB2/Fcα antibody is shown in WO98/02463. U.S. Patent No. 5,821,337 teaches a bispecific anti-ErbB2/anti-CD3 antibody.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. Preferably, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, C<sub>H</sub>2, and C<sub>H</sub>3 regions. It is preferred to have the first heavy-chain constant region (C<sub>H</sub>1) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant affect on the yield of the desired chain combination.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690.

For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

According to another approach described in U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C<sub>H</sub>3 domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science*, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub> fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.*, 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a

V<sub>H</sub> connected to a V<sub>L</sub> by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V<sub>H</sub> and V<sub>L</sub> domains of one fragment are forced to pair with the complementary V<sub>L</sub> and V<sub>H</sub> domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See  
 5 Gruber *et al.*, *J. Immunol.*, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.* *J. Immunol.* 147: 60 (1991).

(vii) *Multivalent Antibodies*

10 A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and  
 15 three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide  
 20 chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)<sub>n</sub>-VD2-(X2)<sub>n</sub>-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two  
 25 (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

30 (viii) *Other amino acid sequence modifications*

Amino acid sequence modification(s) of the anti-PSCA antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the anti-PSCA antibody are prepared by introducing appropriate nucleotide changes into the anti-PSCA antibody nucleic acid, or by peptide synthesis.  
 35 Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the anti-PSCA antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the anti-PSCA antibody, such as changing the number or position of glycosylation sites.



A useful method for identification of certain residues or regions of the anti-PSCA antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells in *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with PSCA antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed anti-PSCA antibody variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an anti-PSCA antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the anti-PSCA antibody molecule include the fusion to the N- or C-terminus of the anti-PSCA antibody to an enzyme (*e.g.* for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the anti-PSCA antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

TABLE 1 Amino Acid Substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile

Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the anti-PSCA antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a

crystal structure of the antigen-antibody complex to identify contact points between the antibody and human PSCA. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylglucosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Nucleic acid molecules encoding amino acid sequence variants of the anti-PSCA antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the anti-PSCA antibody.

It may be desirable to modify the antibody of the invention with respect to effector function, *e.g.* so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp. Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al. Anti-Cancer Drug Design* 3:219-230 (1989).

To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of

an IgG molecule (*e.g.*, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, or IgG<sub>4</sub>) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

(ix) *Screening for antibodies with the desired properties*

5 Techniques for generating antibodies have been described above. One may further select antibodies with certain biological characteristics, as desired.

The growth inhibitory effects of an anti-PSCA antibody of the invention may be assessed by methods known in the art, *e.g.*, using cells which express PSCA either endogenously or following transfection with the PSCA gene. For example, the tumor cell lines and PSCA-transfected cells provided in  
10 Example 2 below may be treated with an anti-PSCA monoclonal antibody of the invention at various concentrations for a few days (*e.g.*, 2-7) days and stained with crystal violet or MTT or analyzed by some other colorimetric assay. Another method of measuring proliferation would be by comparing <sup>3</sup>H-thymidine uptake by the cells treated in the presence or absence of an anti-PSCA antibody of the invention. After antibody treatment, the cells are harvested and the amount of radioactivity incorporated into the DNA quantitated in a  
15 scintillation counter. Appropriate positive controls include treatment of a selected cell line with a growth inhibitory antibody known to inhibit growth of that cell line. Growth inhibition of tumor cells *in vivo* can be determined in various ways such as is described in the Experimental Examples section below (see Example 6). Preferably, the tumor cell is one that over-expresses PSCA. Preferably, the anti-PSCA antibody will inhibit cell proliferation of a PSCA-expressing tumor cell *in vitro* or *in vivo* by about 25-100% compared to  
20 the untreated tumor cell, more preferably, by about 30-100%, and even more preferably by about 50-100% or 70-100%, at an antibody concentration of about 0.5 to 30 µg/ml. Growth inhibition can be measured at an antibody concentration of about 0.5 to 30 µg/ml or about 0.5 nM to 200 nM in cell culture, where the growth inhibition is determined 1-10 days after exposure of the tumor cells to the antibody. The antibody is growth inhibitory *in vivo* if administration of the anti-PSCA antibody at about 1 µg/kg to about 100 mg/kg body  
25 weight results in reduction in tumor size or tumor cell proliferation within about 5 days to 3 months from the first administration of the antibody, preferably within about 5 to 30 days.

To select for antibodies which induce cell death, loss of membrane integrity as indicated by, *e.g.*, propidium iodide (PI), trypan blue or 7AAD uptake may be assessed relative to control. A PI uptake assay can be performed in the absence of complement and immune effector cells. PSCA-expressing tumor cells are  
30 incubated with medium alone or medium containing the appropriate monoclonal antibody at *e.g.*, about 10 µg/ml. The cells are incubated for a 3 day time period. Following each treatment, cells are washed and aliquoted into 35 mm strainer-capped 12 x 75 tubes (1ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 µg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce  
35 statistically significant levels of cell death as determined by PI uptake may be selected as cell death-inducing antibodies.

To screen for antibodies which bind to an epitope on PSCA bound by an antibody of interest, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. This assay can be used to determine if a  
40 test antibody binds the same site or epitope as an anti-PSCA antibody of the invention. Alternatively, or

additionally, epitope mapping can be performed by methods known in the art. For example, the antibody sequence can be mutagenized such as by alanine scanning, to identify contact residues. The mutant antibody is initially tested for binding with polyclonal antibody to ensure proper folding. In a different method, peptides corresponding to different regions of PSCA can be used in competition assays with the test antibodies or with a test antibody and an antibody with a characterized or known epitope.

(x) *Immunoconjugates*

The invention also pertains to therapy with immunoconjugates comprising an antibody conjugated to an anti-cancer agent such as a cytotoxic agent or a growth inhibitory agent.

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above.

Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, maytansinoids, a trichothene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein.

A. Maytansine and maytansinoids

In one preferred embodiment, an anti-PSCA antibody (full length or fragments) of the invention is conjugated to one or more maytansinoid molecules.

Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Patent No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533, the disclosures of which are hereby expressly incorporated by reference.

B. Maytansinoid-antibody conjugates

In an attempt to improve their therapeutic index, maytansine and maytansinoids have been conjugated to antibodies specifically binding to tumor cell antigens. Immunoconjugates containing maytansinoids and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. Liu *et al.*, Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described immunoconjugates comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an *in vivo* tumor growth assay. Chari *et al.* Cancer Research 52:127-131 (1992) describe immunoconjugates in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/*neu* oncogene. The cytotoxicity of the TA.1-maytansinoid conjugate was tested *in vitro* on the human breast cancer cell line SK-BR-3, which expresses  $3 \times 10^5$  HER-2 surface

antigens per cell. The drug conjugate achieved a degree of cytotoxicity similar to the free maytansinoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

5 C. Anti-PSCA antibody-maytansinoid conjugates (immunoconjugates)

Anti-PSCA antibody-maytansinoid conjugates are prepared by chemically linking an anti-PSCA antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. An average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the  
10 function or solubility of the antibody, although even one molecule of toxin/antibody would be expected to enhance cytotoxicity over the use of naked antibody. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed, for example, in U.S. Patent No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytansinoids are maytansinol and maytansinol analogues modified in the aromatic  
15 ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

There are many linking groups known in the art for making antibody-maytansinoid conjugates, including, for example, those disclosed in U.S. Patent No. 5,208,020 or EP Patent 0 425 235 B1, and Chari *et al. Cancer Research* 52: 127-131 (1992). The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-  
20 identified patents, disulfide and thioether groups being preferred.

Conjugates of the antibody and maytansinoid may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as  
25 glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particularly preferred coupling agents include N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (Carlsson *et al., Biochem. J.* 173:723-737 [1978]) and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a  
30 disulfide linkage. See Example 7 below.

The linker may be attached to the maytansinoid molecule at various positions, depending on the type of the link. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. The reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20  
35 position having a hydroxyl group. In a preferred embodiment, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

Calicheamicin

Another immunoconjugate of interest comprises an anti-PSCA antibody conjugated to one or more  
40 calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. For the preparation of conjugates of the calicheamicin family,

see U.S. patents 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, 5,877,296 (all to American Cyanamid Company). Structural analogues of calicheamicin which may be used include, but are not limited to,  $\gamma_1^I$ ,  $\alpha_2^I$ ,  $\alpha_3^I$ , N-acetyl- $\gamma_1^I$ , PSAG and  $\theta_1^I$  (Hinman *et al. Cancer Research* 53: 3336-3342 (1993), Lode *et al. Cancer Research* 58: 2925-2928 (1998) and the aforementioned U.S. patents to American Cyanamid). Another anti-tumor drug that the antibody can be conjugated is QFA which is an antifolate. Both calicheamicin and QFA have intracellular sites of action and do not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody mediated internalization greatly enhances their cytotoxic effects.

#### 10      Other cytotoxic agents

Other antitumor agents that can be conjugated to the anti-PSCA antibodies of the invention include BCNU, streptozocin, vincristine and 5-fluorouracil, the family of agents known collectively LL-E33288 complex described in U.S. patents 5,053,394, 5,770,710, as well as esperamicins (U.S. patent 5,877,296).

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcun, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

20      The present invention further contemplates an immunoconjugate formed between an antibody and a compound with nucleolytic activity (*e.g.* a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

For selective destruction of the tumor, the antibody may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated anti-PSCA antibodies. Examples include At<sup>211</sup>, I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup>, Re<sup>186</sup>, Re<sup>188</sup>, Sm<sup>153</sup>, Bi<sup>212</sup>, P<sup>32</sup>, Pb<sup>212</sup> and radioactive isotopes of Lu. When the conjugate is used for diagnosis, it may comprise a radioactive atom for scintigraphic studies, for example tc<sup>99m</sup> or I<sup>123</sup>, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

30      The radio- or other labels may be incorporated in the conjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as tc<sup>99m</sup> or I<sup>123</sup>, Re<sup>186</sup>, Re<sup>188</sup> and In<sup>111</sup> can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker *et al* (1978) *Biochem. Biophys. Res. Commun.* 80: 49-57 can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunosciintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as

glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari *et al. Cancer Research* 52: 127-131 (1992); U.S. Patent No. 5,208,020) may be used.

Alternatively, a fusion protein comprising the anti-PSCA antibody and cytotoxic agent may be made, *e.g.* by recombinant techniques or peptide synthesis. The length of DNA may comprise respective regions encoding the two portions of the conjugate either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.* avidin) which is conjugated to a cytotoxic agent (*e.g.* a radionucleotide).

(xi) *Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT)*

The antibodies of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as  $\beta$ -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs;  $\beta$ -lactamase useful for converting drugs derivatized with  $\beta$ -lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.



The enzymes of this invention can be covalently bound to the anti-PSCA antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.*, Neuberger *et al.*, *Nature*, 312: 604-608 (1984)).

(xi) *Other antibody modifications*

Other modifications of the antibody are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, 16th edition, Oslo, A., Ed., (1980).

The anti-PSCA antibodies disclosed herein may also be formulated as immunoliposomes. A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.* *J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon *et al.* *J. National Cancer Inst.* 81(19)1484 (1989).

#### IV. Vectors, Host Cells and Recombinant Methods

The invention also provides isolated nucleic acid encoding the humanized anti-PSCA antibody, vectors and host cells comprising the nucleic acid, and recombinant techniques for the production of the antibody.

For recombinant production of the antibody, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(i) *Signal sequence component*

The anti-PSCA antibody of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native anti-PSCA antibody signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader,  $\alpha$  factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

The DNA for such precursor region is ligated in reading frame to DNA encoding the anti-PSCA antibody.

(ii) *Origin of replication*

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 $\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

(iii) *Selection gene component*

Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the anti-PSCA antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, *etc.*

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL-9096).

5 Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding anti-PSCA antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

10 A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, *Nature*, 282:39 (1979)). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics*, 85:12 (1977). The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient  
15 yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

In addition, vectors derived from the 1.6  $\mu$ m circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Alternatively, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg, *Bio/Technology*, 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of  
20 *Kluyveromyces* have also been disclosed. Fleer *et al.*, *Bio/Technology*, 9:968-975 (1991).

(iv) *Promoter component*

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the anti-PSCA antibody nucleic acid. Promoters suitable for use with prokaryotic  
25 hosts include the *phoA* promoter,  $\beta$ -lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan (*trp*) promoter system, and hybrid promoters such as the *tac* promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the anti-PSCA antibody.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich  
30 region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

35 Examples of suitable promoter sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

40 Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2,

isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

5 Anti-PSCA antibody transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are  
10 compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446.  
15 A modification of this system is described in U.S. Patent No. 4,601,978. See also Reyes *et al.*, *Nature* 297:598-601 (1982) on expression of human  $\beta$ -interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

20 (v) *Enhancer element component*

Transcription of a DNA encoding the anti-PSCA antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the  
25 replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the anti-PSCA antibody-encoding sequence, but is preferably located at a site 5' from the promoter.

30 (vi) *Transcription termination component*

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and,  
35 occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding anti-PSCA antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

(vii) *Selection and transformation of host cells*

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

Full length antibody, antibody fragments, and antibody fusion proteins can be produced in bacteria, in particular when glycosylation and Fc effector function are not needed, such as when the therapeutic antibody is conjugated to a cytotoxic agent (e.g., a toxin) and the immunoconjugate by itself shows effectiveness in tumor cell destruction. Full length antibodies have greater half life in circulation. Production in *E. coli* is faster and more cost efficient. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. 5,648,237 (Carter et. al.), U.S. 5,789,199 (Joly et al.), and U.S. 5,840,523 (Simmons et al.) which describes translation initiation region (TIR) and signal sequences for optimizing expression and secretion, these patents incorporated herein by reference. After expression, the antibody is isolated from the *E. coli* cell paste in a soluble fraction and can be purified through, e.g., a protein A or G column depending on the isotype. Final purification can be carried out similar to the process for purifying antibody expressed e.g., in CHO cells.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for anti-PSCA antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, e.g., *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida*; *Trichoderma reesei* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

Suitable host cells for the expression of glycosylated anti-PSCA antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)) ; baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)) ; mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)) ; monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors for anti-PSCA antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

#### (viii) *Culturing the host cells*

The host cells used to produce the anti-PSCA antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

#### (ix) *Purification of anti-PSCA antibody*

When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF

may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human  $\gamma 1$ ,  $\gamma 2$ , or  $\gamma 4$  heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human  $\gamma 3$  (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a  $C_H3$  domain, the Bakerbond ABX<sup>TM</sup>resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE<sup>TM</sup> chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

## V. Pharmaceutical Formulations

Therapeutic formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as acetate, Tris, phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; tonicifiers such as trehalose and sodium chloride; sugars such as sucrose, mannitol, trehalose or sorbitol; surfactant such as polysorbate; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN<sup>TM</sup>, PLURONIC<sup>TM</sup> or polyethylene glycol (PEG). The antibody preferably comprises the antibody at a concentration of between 5-200 mg/ml, preferably between 10-100 mg/ml.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, in addition to the anti-PSCA antibody which internalizes, it may be desirable to include in the one formulation, an additional antibody, *e.g.* a second anti-PSCA antibody which binds a different epitope on PSCA, or an antibody to some other target such as a growth factor that affects the growth of the particular cancer. Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

## VI. Treatment with the Anti-PSCA Antibodies

According to the present invention, the anti-PSCA antibody that internalizes upon binding PSCA on a cell surface is used to treat a PSCA-expressing cancer cell, in particular, bladder and prostate cancer, such as androgen independent prostate cancer or androgen dependent prostate cancer, and associated metastases. A patient may be diagnosed as having androgen independent prostate cancer in that he no longer responds to anti-androgen therapy and the patient diagnosed as having androgen dependent prostate cancer may be one who responds to anti-androgen therapy. The cancer will generally comprise PSCA-expressing cells, such that the anti-PSCA antibody is able to bind thereto. While the cancer may be characterized by overexpression of the PSCA molecule, the present application further provides a method for treating cancer which is not considered to be an PSCA-overexpressing cancer.

To determine PSCA expression in the cancer, various diagnostic assays are available. In one embodiment, PSCA overexpression may be analyzed by immunohistochemistry (IHC). Paraffin embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a PSCA protein staining intensity criteria as follows:



Score 0 no staining is observed or membrane staining is observed in less than 10% of tumor cells.

Score 1+ a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.

5 Score 2+ a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

Score 3+ a moderate to strong complete membrane staining is observed in more than 10% of the tumor cells.

Those tumors with 0 or 1+ scores for PSCA expression may be characterized as not overexpressing PSCA, whereas those tumors with 2+ or 3+ scores may be characterized as overexpressing PSCA.

Alternatively, or additionally, FISH assays such as the INFORM™ (sold by Ventana, Arizona) or  
10 PATHVISION™ (Vysis, Illinois) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of PSCA overexpression in the tumor.

PSCA overexpression or amplification may be evaluated using an *in vivo* diagnostic assay, *e.g.* by administering a molecule (such as an antibody) which binds the molecule to be detected and is tagged with a detectable label (*e.g.* a radioactive isotope or a fluorescent label) and externally scanning the patient for  
15 localization of the label.

Currently, depending on the stage of the cancer, prostate cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, androgen deprivation (*e.g.*, hormonal therapy), and chemotherapy. Anti-PSCA antibody therapy may be especially desirable in elderly patients who do not tolerate the toxicity and side effects of chemotherapy well, in  
20 metastatic disease where radiation therapy has limited usefulness, and for the management of prostatic carcinoma that is resistant to androgen deprivation treatment. The tumor targeting and internalizing anti-PSCA antibodies of the invention are useful to alleviate PSCA-expressing cancers, *e.g.* prostate and bladder cancers upon initial diagnosis of the disease or during relapse. For therapeutic applications, the anti-PSCA antibody can be used alone, or in combination therapy with, *e.g.*, hormones, antiangiogens, or radiolabelled  
25 compounds, or with surgery, cryotherapy, and/or radiotherapy, notably for prostate cancers, also particularly where shed cells cannot be reached. Anti-PSCA antibody treatment can be administered in conjunction with other forms of conventional therapy, either consecutively with, pre- or post-conventional therapy. Chemotherapeutic drugs such as taxotere® (docetaxel), taxol® (paclitaxel), estramustine and mitoxantrone are used in treating metastatic and hormone refractory prostate cancer, in particular, in good risk patients. In  
30 the present method of the invention for treating or alleviating cancer, in particular, androgen independent and/or metastatic prostate cancer, the cancer patient can be administered anti-PSCA antibody in conjunction with treatment with the one or more of the preceding chemotherapeutic agents. In particular, combination therapy with paclitaxel and modified derivatives (see, *e.g.*, EP0600517) is contemplated. The anti-PSCA antibody will be administered with a therapeutically effective dose of the chemotherapeutic agent. In another  
35 embodiment, the anti-PSCA antibody is administered in conjunction with chemotherapy to enhance the activity and efficacy of the chemotherapeutic agent, *e.g.*, paclitaxel. The Physicians' Desk Reference (PDR) discloses dosages of these agents that have been used in treatment of various cancers. The dosing regimen and dosages of these aforementioned chemotherapeutic drugs that are therapeutically effective will depend on the particular cancer being treated, the extent of the disease and other factors familiar to the physician of skill  
40 in the art and can be determined by the physician.

In one particular embodiment, an immunoconjugate comprising the anti-PSCA antibody conjugated with a cytotoxic agent is administered to the patient. Preferably, the immunoconjugate bound to the PSCA protein is internalized by the cell, resulting in increased therapeutic efficacy of the immunoconjugate in killing the cancer cell to which it binds. In a preferred embodiment, the cytotoxic agent targets or interferes with the nucleic acid in the cancer cell. Examples of such cytotoxic agents are described above and include  
5 maytansinoids, calicheamicins, ribonucleases and DNA endonucleases.

The anti-PSCA antibodies or immunoconjugates are administered to a human patient, in accord with known methods, such as intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial,  
10 intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the antibody is preferred.

Other therapeutic regimens may be combined with the administration of the anti-PSCA antibody. The combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time  
15 period while both (or all) active agents simultaneously exert their biological activities. Preferably such combined therapy results in a synergistic therapeutic effect.

It may also be desirable to combine administration of the anti-PSCA antibody or antibodies, with administration of an antibody directed against another tumor antigen associated with the particular cancer.

In another embodiment, the antibody therapeutic treatment method of the present invention involves  
20 the combined administration of an anti-PSCA antibody (or antibodies) and one or more chemotherapeutic agents or growth inhibitory agents, including co-administration of cocktails of different chemotherapeutic agents. Chemotherapeutic agents include estramustine phosphate, prednimustine, cisplatin, 5-fluorouracil, melphalan, cyclophosphamide, hydroxyurea and hydroxyureataxanes (such as paclitaxel and doxorubicin) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be  
25 used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992).

The antibody may be combined with an anti-hormonal compound; *e.g.*, an anti-estrogen compound such as tamoxifen; an anti-progesterone such as onapristone (see, EP 616 812); or an anti-androgen such as  
30 flutamide, in dosages known for such molecules. Where the cancer to be treated is androgen independent cancer, the patient may previously have been subjected to anti-androgen therapy and, after the cancer becomes androgen independent, the anti-PSCA antibody (and optionally other agents as described herein) may be administered to the patient.

Sometimes, it may be beneficial to also co-administer a cardioprotectant (to prevent or reduce  
35 myocardial dysfunction associated with the therapy) or one or more cytokines to the patient. In addition to the above therapeutic regimens, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy, before, simultaneously with, or post antibody therapy. Suitable dosages for any of the above co-administered agents are those presently used and may be lowered due to the combined action (synergy) of the agent and anti-PSCA antibody.

40 For the prevention or treatment of disease, the dosage and mode of administration will be chosen by the physician according to known criteria. The appropriate dosage of antibody will depend on the type of

disease to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Preferably, the antibody is administered by intravenous infusion or by subcutaneous injections. Depending on the type and severity of the disease, about 1 µg/kg to about 50 mg/kg body weight (*e.g.* about 0.1-15mg/kg/dose) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A dosing regimen can comprise administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the anti-PSCA antibody. However, other dosage regimens may be useful. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. The progress of this therapy can be readily monitored by conventional methods and assays and based on criteria known to the physician or other persons of skill in the art.

Aside from administration of the antibody protein to the patient, the present application contemplates administration of the antibody by gene therapy. Such administration of nucleic acid encoding the antibody is encompassed by the expression "administering a therapeutically effective amount of an antibody". See, for example, WO96/07321 published March 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells; *in vivo* and *ex vivo*. For *in vivo* delivery the nucleic acid is injected directly into the patient, usually at the site where the antibody is required. For *ex vivo* treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes which are implanted into the patient (see, *e.g.* U.S. Patent Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. A commonly used vector for *ex vivo* delivery of the gene is a retroviral vector.

The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). For review of the currently known gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256:808-813 (1992). See also WO 93/25673 and the references cited therein.

## VII. Articles of Manufacture and Kits

Another embodiment of the invention is an article of manufacture containing materials useful for the treatment of anti-PSCA expressing cancer, in particular prostate cancer and bladder cancer. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of

materials such as glass or plastic. The container holds a composition which is effective for treating the cancer condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-PSCA antibody of the invention. The label or package insert indicates that the composition is used for treating prostate cancer, androgen independent prostate cancer, or androgen dependent prostate cancer, or bladder cancer. The label or package insert will further comprise instructions for administering the antibody composition to the cancer patient. Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution.

It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Kits are also provided that are useful for various purposes, e.g., for PSCA cell killing assays, for purification or immunoprecipitation of PSCA from cells. For isolation and purification of PSCA, the kit can contain an anti-PSCA antibody coupled to beads (e.g., sepharose beads). Kits can be provided which contain the antibodies for detection and quantitation of PSCA *in vitro*, e.g. in an ELISA or a Western blot. As with the article of manufacture, the kit comprises a container and a label or package insert on or associated with the container. The container holds a composition comprising at least one anti-PSCA antibody of the invention. Additional containers may be included that contain, e.g., diluents and buffers, control antibodies. The label or package insert may provide a description of the composition as well as instructions for the intended *in vitro* or diagnostic use.

## VIII. Experimental Examples

The above and other features of the invention will now be described more particularly with reference to the accompanying figures and pointed out in the claims. The particular embodiments described below are provided by way of illustration and are not meant to be construed as a limitation on the scope of the invention. It will be apparent to one of ordinary skill in the art that many modifications can be made to the present invention without departing from the spirit or essential characteristics of the invention.

### Example 1

The preparation and characterization of the anti-PSCA monoclonal antibodies are described.

#### I. Materials and Methods

Hybridomas producing murine and human anti-PSCA monoclonal antibodies (Mabs) were generated. The fusion partner was the mouse myeloma line P3X63Ag8.653 (Kearney, J.F. et al., J. Immunology 123:1548-1550, 1979).

Table 2A summarizes the immunization strategies and shows the nature of the antigen used for immunization, the adjuvant, dosage, route of administration, dosing schedule and host animal used. Table 2B summarizes the characteristics of the anti-PSCA Mabs as determined by various assays. The antibody names herein are derived from the first 3-4 characters of the hybridoma clone producing the antibody (see Table 2B). The first 3-4 characters before the period in the clone name indicate the parental clone followed after the first period by the name of further subclones, if any. Alternatively, the anti-PSCA antibodies herein are also referred to by their designated ascites number. Except where muPSCA is indicated, human (hu) PSCA

by itself or fused to a gD tag (gDPSCA) or histidine tag (his-PSCA), was used. Human or murine PSCA expressed in E.coli was histidine-tagged. Unless otherwise indicated, PSCA expressed in E.coli was refolded, as described below, for immunization. gDPSCA-CHO refers to soluble PSCA expressed in and purified from CHO cells. Antibodies prepared from Xenomice (Abgenix, Fremont, California) are fully human antibodies.

For the hu PSCA nucleotide and amino acid sequences, refer to WO 99/14328 (Genentech; PRO232 protein, nucleotide sequence in Fig. 1; amino acid sequence in Fig. 2); U.S. Patent 5,856,136 issued January 5, 1999 (Incyte; SCAH2 SEQ ID4); or Fig. 2 on page 1737 of Reiter et al. *Proc. Nat. Acad. Sci.* 95:1735-1740 (1998). For convenience, the 123 amino acid sequence and the coding sequence of the cDNA of PSCA are provided herein as SEQ ID NO. 1 and SEQ ID NO. 2, respectively. The numbering of the amino acid sequence referred to herein will be according to that shown in SEQ ID NO. 1 which is the same as in Reiter et al. (1998).

#### Cell Lines and Transfections

A plasmid vector encoding hu PSCA or gDPSCA was transfected into the following cells: NIH 3T3 cells expressing Ras (Ras 3T3); and CHO; HCT116. For expression in E. coli, the sequence encoding hu PSCA amino acids 10-101 was used (fused to a His tag), with the GPI anchor sequence removed. For expression in 3T3 cells, the N-terminal signal sequence (aa 1-20) of PSCA was replaced by the signal sequence of the gD tag followed by 25 amino acids of the mature gD.

#### Binding Assays

The anti-PSCA antibodies were analyzed for binding to PSCA on cells by Fluorescence Activated Cell Sorter (FACS). Purified murine anti-PSCA 6B8.1D7.2B3 (#2761) antibody and fully human anti-PSCA 10C5.6E4.6D1 (#2910) antibody from hybridoma supernatant were analyzed for cell surface binding by FACS. Confluent adherent stable CHO cells transfected with huPSCA with a gD tag at its N-terminus were washed with PBS and detached using Cell Dissociation Solution (Sigma). Cells were incubated with the anti-PSCA antibodies at various concentrations (1-10  $\mu$ g/ml) and washed with PBS prior to incubation with a FITC conjugated secondary antibody. Cells were resuspended in PBS containing 1% FBS or 1% formaldehyde for cell fixation. The binding of antibodies to the cell surface was then analyzed on a FACScan.

To assess the ability of the Mabs to capture PSCA in solution, an assay was developed in which each antibody was either directly coated onto microtiter plates or bound to an anti-mouse IgG specific to the Fc region, previously coated on the plate. Biotinylated PSCA antigen (1  $\mu$ g/ml) was then added to the plate prior to incubation with streptavidin-HRP. OPD was used as a substrate and plates were read at 492nm.

To verify the binding of Mab #2403 to PSCA expressed on the surface of transfected cells, a cold competition binding assay was performed using  $^{125}$ I-labeled Mab #2403 (lactoperoxidase method). PC3.gD.hu PSCA cells ( $1 \times 10^5$ ) were plated in a 24 well dish and incubated in medium with various concentrations of unlabeled Mab 2403 and a constant amount of  $^{125}$ I-Mab 2403 for 16 hours at 4°C. Unbound antibody was removed and cells were washed with ice-cold medium. After solubilization of the cells in 8M Urea/3M glacial acetic acid, the amount of radioactivity bound was determined using a gamma

counter. The  $IC_{50}$  value was calculated from a four parameter fit calculation of the curve where  $m_3$  represent the  $IC_{50}$ . The results of this assay are plotted in Figure 4.

A direct ELISA was performed to determine the binding activity of the #2761 (6B8) antibody. HuPSCA and murine PSCA (mPSCA) were coated overnight at  $1\mu\text{g/ml}$  onto a microtiter plate (Nunc). The anti-PSCA Mab and controls were added to the antigen-coated wells and incubated at room temperature. Unbound antibody was washed off and a horseradish peroxidase (HRP)-conjugated secondary antibody was added to the wells and incubated with the antibodies at room temperature. OPD substrate solution was finally added and concentrated sulfuric acid was used to stop the reaction. The plate was read at 492 nm using a microtiter plate reader.

Epitope groupings were determined by competitive ELISA. Each antibody was biotinylated and tested for binding to PSCA coated on plates, in the presence or absence of an excess of each unlabeled anti-PSCA Mab. Streptavidin-HRP was then added to the plates followed by peroxidase substrate. Decrease (at least 50%) or lack of binding of biotinylated Mabs to PSCA indicated that both cold and biotinylated antibodies bound to the same (or proximal) epitope on PSCA.

#### Purification and refolding of His-tagged proteins produced in *E. coli*

The following procedure has been adapted for use on proteins produced from 0.5 to 1L fermentations of *E. coli* expressing His-tagged proteins. It can easily be scaled to much larger amounts.

*E. coli* paste from 0.5 to 1L fermentations ( 6-10 gm pellets) was resuspended in 10 volumes (w/v) in 7M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate were added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution was stirred overnight at  $4^{\circ}\text{C}$ . This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution was centrifuged at 40 K rpm in a Beckman Ultracentrifuge for 30 min. The supernatant was diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. Depending on the expression of the desired protein, 30-50 mls of the clarified extract were loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column was washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein was eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein were pooled and stored at  $4^{\circ}\text{C}$ . Protein concentration was estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins are usually refolded by diluting sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. In the case of PSCA, 3.5M Urea and 1 mM cysteine were used instead of 2.5 M Urea and 5 mM cysteine. Refolding volumes were chosen so that the final protein concentration would be between 50 to 100 micrograms/ml. The refolding solution was stirred gently at  $4^{\circ}\text{C}$  for 12-36 hours. The refolding reaction was quenched by the addition of trifluoroacetic acid (TFA) to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution was filtered through a 0.22 micron filter and acetonitrile added to 2-10% final concentration. The refolded protein was chromatographed on a Vydac C4 reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10% to 80%. Aliquots of fractions with A280 absorbance were analyzed on SDS polyacrylamide gels and

fractions containing homogeneous refolded protein were pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded protein were pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins were usually formulated into 20 mM Hepes, pH 6.8 with 0.14 M NaCl and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered. Occasionally, proteins with isoelectric points in the pH range of 6-7.5 would be formulated in a buffer of 1 mM HCl, 0.14 M NaCl to maintain solubility. Formulated proteins were aliquoted and frozen and stored at  $-80^{\circ}\text{C}$ . The protein was subsequently characterized by SDS gels, amino acid analysis for quantitation of protein, evaluated for endotoxin levels by an endotoxin assay, N-terminal protein sequencing to establish identity of protein, mass spectrometry to establish integrity of protein and other analytical procedures as necessary or desired.

#### Immunoglobulin Sequences and Constructs

Purified antibodies were subjected to amino acid sequencing using established methods. The N-terminal sequence was obtained and from that, primers were designed to clone out the Ig variable region (V) sequences by RT-PCR. The DNA fragment encoding the  $V_L$  or  $V_H$  region was cloned into a vector. Sequencing was performed using established methods, initially with primers to the vector. From the initial nucleotide sequence of the antibody that was obtained, primers specific to the antibody sequence were designed to obtain the remaining sequence.

The chimeric full length and Fab mouse-human anti-PSCA antibodies whose sequences are shown in Figure 13 were constructed by joining the murine  $V_L$  and  $V_H$  region domain sequences to the human C $\kappa$  and C $\gamma$ 1 sequences. The murine antibody V region fragments were cloned into vectors as restriction site cassettes which can be readily excised and ligated into preconstructed vectors containing human L and H constant regions of different isotypes to express chimeric mouse-human antibodies with PSCA specificity. The Fab chimeric chains were expressed from the vector called vegfchim derived from pUC119:Ig vector which is a pRK vector (described in EP 307,247).

Figure 12 shows the amino acid sequences of the light and heavy chain variable region domains ( $V_L$  and  $V_H$ ) of the antibodies from hybridoma clones 6F8.2F4, Asc# 2395 (SEQ ID NO. 3 & NO. 4); 5F2.4H4.1E3, Asc# 2403 (SEQ ID NO. 5 & NO.6); 6B8.1D7.2B3, Asc#2761 (SEQ ID NO. 7 & NO. 8), and  $V_H$  of the antibody from clone 8D11.2E9 (Asc# 2399 (SEQ ID NO. 9). Figure 13 shows the amino acid sequences of the full length chimeric 2403 IgG including the signal peptide sequences (L chain is SEQ ID NO. 10; H chain is SEQ ID NO. 11) and the sequences of chimeric 6B8 Fab (L chain is SEQ ID NO. 12; H chain is SEQ ID NO. 13). Chimeric antibody 2403 was made by fusing the murine  $V_L$  and  $V_H$  sequences of Mab 5F2.4H4.1E3 (#2403) with human immunoglobulin C $\kappa$  and C $\gamma$ 1 sequences, respectively, in the mammalian cell expression vector, pRK. The construction of the pRK5 expression vector is disclosed in EP 307,247 published March 13, 1989. The pRK vectors encoding the chimeric 2403 H and L chains have been deposited with the ATCC as DNA pRK-2403H and DNA pRK-2403L (see X below). Mouse  $V_H$  was joined

to human C $\gamma$ 1 at sequential residue # Pro122 (denoted by / in sequence in FIG. 13). Mouse V<sub>L</sub> was joined to human C<sub>L</sub> at sequential residue # Arg113. The 2403 V<sub>H</sub> insert can be excised from pRK-2403H as an EcoRI-ApaI fragment or a ClaI-ApaI fragment of 430 bp. The 2403 V<sub>L</sub> was cloned into pRK-2403L as an EcoRI-CpoI fragment of 400 bp fragment. The pRK vectors encoding the chimeric 2716 H and L chains have been deposited with the ATCC as DNA pRK-2761H and DNA pRK-2761L (see X below). Likewise, the 2716 V<sub>L</sub> has been cloned into pRK-2761L as an EcoRI-CpoI fragment of 400 bp fragment. The 2761 V<sub>H</sub> insert can be excised from pRK-2761H as an EcoRI-ApaI fragment or a ClaI-ApaI fragment of 430 bp.

Chimeric 6B8 Fab L and H chains were made by fusing the murine V<sub>L</sub> and V<sub>H</sub> sequences of Mab 6B8.1D7.2B3 with human immunoglobulin C $\kappa$ , and C<sub>H</sub>1 domain of IgG1 sequences, respectively, in the vector called vegf4chim, and expressed in *E. coli*. The vegf4chim vector has an amp<sup>R</sup> selectable marker and the antibody sequences are operably linked to and expressed under, the alkaline phosphatase promoter.

These V region cassettes can be excised and ligated to constant region domains of the different human immunoglobulin isotypes to produce chimeras with different Fc properties. To produce anti-PSCA antibodies lacking effector functions such as ADCC, complement binding activity or Fc receptor binding, the murine V<sub>H</sub> regions are fused to the human C $\gamma$ 2 or C $\gamma$ 4 constant region sequences.

## II. Results

Table 2B summarizes the characteristics of the anti-PSCA antibodies as determined by the assays herein. In Table 2B, hu PSCA-*E. coli* refers to his-tagged PSCA expressed in *E. coli*; the protein was purified from the bacteria and then refolded as described above; + formalin indicates that formalin fixed cells were used as immunogen; prk5.hu PSCA refers to the naked cDNA carrying the hu PSCA gene in pRK5 plasmid. Xenomice (Abgenix, Fremont, California) are mice which have been genetically engineered to replace their endogenous murine Ig locus with the human germline immunoglobulin locus such that they make human antibodies. Human antibodies to PSCA are made by immunizing the Xenomice with *E. coli* expressed PSCA, intraperitoneally (i.p.) or via the footpad.

All anti-PSCA Mabs tested produced a significant shift in staining of the PSCA-transfected cells as assayed by FACS. Figure 1 (Mab 5F2) and Figure 2 (Mab 10C5) show representative staining results. Cells incubated with FITC-conjugated secondary antibody alone was used as negative control; these cells did not show any antibody binding activity. The anti-gD Mab 1766 gD, used as a positive control, showed significant binding to CHO cells expressing gD-huPSCA. Hybridoma supernatant containing the fully human Mab 10C5.6E4.6D1 also demonstrated strong binding to the cell surface. Murine anti-PSCA Mab 5F2 (#2403) was used as a positive control in this experiment and media as a negative control.

Murine Mab 6B8 (#2761) was tested for its ability to bind to various soluble forms of PSCA immobilized onto a plate and the results are shown in Figure 3. Mab 6B8 generated against denatured PSCA protein, demonstrated stronger affinity for the refolded huPSCA than for nonrefolded huPSCA. The Mab was also shown to bind to gD-huPSCA CHO soluble antigen, but with less affinity as compared to refolded and nonrefolded huPSCA. However, there was no binding to an irrelevant antigen TGF- $\beta$  and murine PSCA, suggesting that the antibody was specific to huPSCA.

From the cold competition binding assay (see Figure 4 and table immediately below), the binding affinity for the Mab 2403 was determined to be 12.9 nM.



5

$y = ((m1-m4)/(1+(m0/m3)^{m2}))+m4$		
	Value	Error
m1	40650	657.34
m2	1.0449	0.074328
m3	12.977	1.0636
m4	1865	1102.9
Chisq	1.9794e+06	NA
R	0.99921	NA

TABLE 2A

CLONE	MAbs ASC#	ANTIGEN	ADJUV.	DOSE	ROUTE	SCHEDULE	HOST
10E3.5A7.1A7	2359	hisPSCA - E. coli	Ribi	5 ug	FP X 8	2/week	Balb/c
6C3.4G12.1B7	2360	hisPSCA - E. coli	"	"	"	"	Balb/c
6F8.2F4	2395	hisPSCA - E. coli	Ribi	"	FP X 6	2/week	Balb/c
10A1.2F11	2396	hisPSCA - E. coli	"	"	"	"	Balb/c
2F2.2G7	2397	hisPSCA - E. coli	"	"	"	"	Balb/c
5B12.1C9	2398	hisPSCA - E. coli	"	"	"	"	Balb/c
8D11.2E9	2399	hisPSCA - E. coli	"	"	"	"	Balb/c
5A5.1D11	2400	hisPSCA - E. coli	"	"	"	"	Balb/c
2D2.1B12	2401	hisPSCA - E. coli	"	"	"	"	Balb/c
5F2.4H4.1E3	2403	hisPSCA - E. coli	"	"	"	"	Balb/c
7A6.1H4.2A5	2405	hisPSCA - E. coli	"	"	"	"	Balb/c
7B9.2C1.2H4.1D12	2429	hisPSCA - E. coli	"	"	"	"	Balb/c
12C5.1F10	2402	hisPSCA - E. coli	Ribi	"	FP X 7	2/week	Balb/c
2A4.3G2	2404	hisPSCA - E. coli	"	"	"	"	Balb/c
7B11.3H5.5C2	2430	hisPSCA - E. coli	"	"	"	"	Balb/c
N/A	N/A	hisPSCA - E. coli	Ribi	"	FP X 6	2/week	Balb/c
N/A	N/A	gDPSCA Ras 3T3	none	tumor	SQ	NA	Balb/c
N/A	N/A	gDPSCA - CHO membranes	Ribi	ND	FP X 6	1/week	Balb/c
5G1.1E5 (S/N)	N/A	hisPSCA - E. coli/formalin	Ribi	5 ug	FP X 10	2/week	Balb/c
N/A	N/A	gDPSCA - CHO	Ribi	5 ug	FP X 10	1/week	Xenomice
N/A	N/A	hisPSCA - E. coli	Ribi	5 ug	FP X 10	1/week	Xenomice
N/A	N/A	his Mu PSCA - E. coli	Ribi	10 ug	FP X 10	1/week	Lewis rats
N/A	N/A	hisPSCA - E. coli	Ribi	5 ug	FP X 5	1/week	Xenomice
N/A	N/A	hisPSCA - E. coli	Ribi	10 ug	IP X 5	1/week	Xenomice
4F9.1E8.1C2 (S/N)	2760	hisPSCA - E. coli/nonrefolded	Ribi	5 ug	FP X 5	1/week	Balb/c
6B8.1D7.2B3	2761	hisNonrefolded huPSCA - E. coli	Ribi	5 ug	FP X 5	1/week	Balb/c
3F9.1D4.2F1 (S/N)	2759	gDPSCA - CHO	Ribi	5 ug	FP X 10	2/week	Balb/c
2B12.1H6.1F9 (S/N)	2758	gDPSCA - CHO	Ribi	5 ug	FP X 11	2/week	Balb/c
N/A	N/A	his Mu PSCA - E. coli	Ribi	10 ug	FP X 5	1/week	Lewis rats
N/A	N/A	PSCA/GST - E. coli	Ribi	5 ug	FP X 8	1/week	Balb/c
NO TITERS	N/A	hPSCA-1/KLH (peptide)	Ribi	5 ug	FP X 7	1/week	Balb/C
NO TITERS	N/A	mPSCA-1/KLH (peptide)	Ribi	5 ug	FP X 7	1/week	Lewis rats
2 PARENTAL S/N	N/A	gDPSCA - HCT116	none	10 e6	FP X 10	1/week	Balb/c
N/A	N/A	gDPSCA - HCT116/formalin	none	10 e6	FP X 10	1/week	Balb/c
NO TITERS	N/A	prk5.hu.PSCA (cDNA)	none	100 ug	IM X 2	2/month	Balb/c

pending	pending	HCT116gDPSCA	none	10 e6	FP X 10	1/week	XenoMice
N/A	N/A	his Mu PSCA -E. coli	Ribi	10 ug	FP X 5	1/week	Lewis Rats
N/A	N/A	hisPSCA - E. coli/nonrefolded	Ribi	5 ug	FP X 5	1/week	XenoMice
N/A	N/A	PSCA/GST - E. coli	Ribi	5 ug	FP X 6	1/week	Balb/c
10C5.6E4.6D1 (S/N)	2910	hisPSCA-E.coli	Ribi	5 ug	IP X5	1/week	XenoMice
pending	pending	hisPSCA - E.coli/nonrefolded	Ribi	5 ug	FP X 5	1/week	XenoMice
pending	pending	his Mu PSCA - E. coli	Ribi	10 ug	FP X 5	1/week	Lewis Rats
pending	pending	PC3gDmPSCA Cells	none	10 X 106	FP X 10	1/week	Lewis Rats

TABLE 2B

CLONE	mAbs Asc#	ANTIGEN	SPECIES OR STRAIN	ISOTYPE	CAPTURE	FACS	IHC	EPITOPE	TUMOR TARGET	INTERNALIZE
10E3.5A7.1A7	2359	hu PSCA-E Coli	Balb/C	IgG1	Yes	+/-	+	E	ND	Yes
6C3.4G12.1B7	2360	hu PSCA-E Coli	Balb/C	IgG2a	Yes	+	-	B	ND	Yes
6F8.2F4	2395	hu PSCA-E Coli	Balb/C	IgG2b	Yes	++	-	A	Yes	Yes
10A1.2F11	2396	hu PSCA-E Coli	Balb/C	IgG2b	Yes	+	-	A	ND	ND
2F2.2G7	2397	hu PSCA-E Coli	Balb/C	IgG2b	No	-	+	ND	ND	ND
5B12.1C9	2398	hu PSCA-E Coli	Balb/C	IgG2b	Yes	-	+/-	C	ND	ND
8D11.2E9	2399	hu PSCA-E Coli	Balb/C	IgG2b	Yes	++	-	A	Yes	Yes
5A5.1D11	2400	hu PSCA-E Coli	Balb/C	IgG2a	Yes	+	-	ND	ND	ND
2D2.1B12	2401	hu PSCA-E Coli	Balb/C	IgG2a	Yes	-	-	D	ND	ND
5F2.4H4.1E3	2403	hu PSCA-E Coli	Balb/C	IgG2b	Yes	++	-	A	Yes	Yes
7A6.1H4.2A5	2405	hu PSCA-E Coli	Balb/C	IgG2a	Yes	+	-	A	ND	ND
7B9.2C1.2H4.1D12	2429	hu PSCA-E Coli	Balb/C	IgG2a	Yes	-	-	F	ND	ND
12C5.1F10	2402	hu PSCA-E Coli	Balb/C	IgG2b	Yes	+/-	-	A	ND	ND
2A4.3G2	2404	hu PSCA-E Coli	Balb/C	IgG2b	No	-	-	ND	ND	ND
7B11.3H5.5C2	2430	hu PSCA-E Coli	Balb/C	IgG1	Yes	+/-	+/-	ND	ND	ND
N/A	N/A	hu gDPSCA Ras 3T3	Balb/C							
N/A	N/A	hu PSCA-CHO membrane	Balb/C							
5G1.1E5	N/A	hu PSCA-E Coli + formalin	Balb/C	IgM	?	?	-			
N/A	N/A	hu gDPSCA - CHO	Xenomice	N/A	N/A	N/A	N/A			
N/A	N/A	hu PSCA-E Coli	Xenomice	N/A	N/A	N/A	N/A			
pending	N/A	muPSCA-E.coli	Lewis rats	IgG2a	Yes	-	-			
4F9.1E8.1C2 (S/N)	2760	hu PSCA-E Coli *	Balb/C	IgG2a	Yes	++	++		ND	
6B8.1D7.2B3	2761	hu PSCA-E Coli *	Balb/C	IgG2a	Yes	++	+++	B	Yes	Yes
3F9.1D4.2F1 (S/N)	2759	gDPSCA-CHO	Balb/C	IgG1	Yes	++	-	A	ND	
2B12.1H6.1F9 (S/N)	2758	gDPSCA-CHO	Balb/C	IgG1	Yes	++	+	A	ND	
2 parental S/N		gDPSCA-HCT116 cells	Balb/C	IgM		+				
pending		gDPSCA-HCT116 /formalin	Balb/C							
pending		gDPSCA-HCT116 cells	Xenomice							

no titers		prk5.hu PSCA (cDNA)	Balb/C	IgG2	Yes	++	+		
10C5.6E4.6D1(S/N)	2910	hu PSCA-E.coli	Xenomice						
N/A		hu PSCA-E.coli *	Xenomice						
pending		muPSCA-E.coli	Lewis rats						
pending		gDmuPSCA-PC3 cells	Lewis rats						

S/N = supernatant

IP = intraperitoneal

FP = via footpad

muPSCA = murine PSCA

ND = not determined

Asc# = Ascites #

IHC = immunohistochemistry

hu PSCA = human PSCA

\*nonrefolded huPSCA

### Example 2

This example demonstrated the binding and localization of anti-PSCA antibodies 2395 (6F8), 2399 (8D11), 2403 (5F2), 2761 (6B8) [ascites number followed by antibody no. in parenthesis; see Table 2B] to PSCA expressing tumor cells (MCF-7Her2gdPSCA and HCT-gdPSCA) *in vivo*. By labeling antibodies with the fluorescent dye, Cy3, antibody binding can be visualized by fluorescence microscopy. This technology was well established using Cy3 labeled Herceptin (anti-Her2 monoclonal antibody) in mice bearing Her2-expressing tumors. *In vivo* binding of the anti-PSCA antibodies were tested on tumor cells transfected with human gd-linked PSCA. Control antibodies included Cy3 labeled Herceptin as a positive control for the MCF-7Her2gdPSCA cells. Additionally, a Cy3 labeled anti-gd antibody, with very good binding activity, was used as a positive control for the PSCA antibodies. Transfected PSCA is gd linked, thus the level of gd protein directly correlates with PSCA protein expression. Cell lines not transfected with gdPSCA were used as negative controls.

#### I. Materials and Methods

##### Cell lines

HCT-116 (ATCC CCL-247) is a human colon tumor cell line; HCT-47 is a PSCA expressing clone derived from transfecting the parental HCT-116. MCF-7 (ATCC HTB-22; Soule, D. G. et al., J. Natl. Cancer Inst., 51:1409-1416, 1973) is a human breast tumor cell line which was previously transfected with and expresses Her2 (Benz et al. *Breast Cancer Research and Treatment* 24: 85-95 (1992)). PC3 (ATCC CRL-1435) is a human prostatic carcinoma cell line (Kaighn et al. *Invest. Urol.*, 17: 16-23, 1979). These cells were transfected with human PSCA (huPSCA) or with gD-huPSCA. gD or gd refers to the Herpes Simplex virus glycoprotein D epitope tag. In the construction of gD-huPSCA, the signal sequence of PSCA was replaced with the sequence encoding the gD epitope tag; the gD-PSCA sequence was cloned into pRK vector. Her2 expression on the cell surface served as a control since Her2 antibody (HERCEPTIN®) is known to be able to localize to Her2-expressing tumor cells *in vivo*. From these transfections, human PSCA-expressing tumor cells were obtained.

##### Antibody preparation

Refer to Table 2B for a list of the murine monoclonal anti-PSCA antibodies used in these experiments. The antibody names are derived from the first 3-4 characters of the clone producing the antibody. All antibodies were labeled using FluoroLink Cy3 monofunctional dye (Amersham Pharmacia, PA23001) according to the manufacturer's directions. Antibodies for the labeling reaction were in 0.1 M Sodium Carbonate pH 9.3 at a concentration of 1 mg/ml. One ml of the 1 mg/ml of antibody stock was added to the tube with Cy3 labeling mix, mixed thoroughly, and allowed to incubate at room temperature for 30 min on a shaker. Samples were wrapped in tin foil to reduce light exposure. NAP 10 columns (Amicon) were equilibrated with PBS and when the conjugation reaction was done, the reaction solution was added to the column. 1.5 ml PBS were added and the flow through was collected. Spectrophotometer readings were taken at A552 and A280. Protein concentration and dye to protein ratios were calculated as follows:

AB concentration:  $\{[A280 - (0.08 \times A552)] \times \text{dilution factor}\} / 1.4 = \text{mg/ml}$

Dye to Protein ratio:  $[1.13 \times (A552)] / [A280 - (0.08 \times A552)]$

After labeling, all antibodies were checked for binding by FACS analysis (see Table 2B).

### Tumor inoculations and growth

NCR nude female mice were implanted with Estrogen pellets and then inoculated subcutaneously with 20 million cells of MCF-7Her2gdPSCA or 20 million cells of non-transfected MCF-7 cell line as a negative control. Male NCR nude mice were inoculated subcutaneously with 5 million HCT-gdPSCA cells. The tumors are injected subcutaneously, so they are situated on the flank of the mouse. Using a caliper with a digital read out, tumor measurements were taken twice weekly until tumor volume reached 100 to 500 mm<sup>3</sup>.

### 10 Cy3 Antibody injection and tissue collection

Once tumors reached at least 150 mm<sup>3</sup> in volume, mice were injected IP with 10 mg/kg of one of the three Cy3 labeled anti-PSCA antibodies (6F2, 8D11, 5F2), a Cy3 anti-gd antibody, or Cy3 labeled HERCEPTIN. Twenty four hours later, mice were anesthetized and perfused with 1% paraformaldehyde. Tumor tissue and other organs were collected and frozen in OCT (Tissue-Tek O.C.T. Compound, Sakura  
15 Finetek U.S.A. Inc., Torrance CA 90504) cryoprotectant for antibody localization by fluorescence microscopy. Similar experiments were also carried out with Cy3 labeled 6B8 antibody.

### Fluorescence microscopy

Frozen samples were sectioned into 5 um sections and fluorescent mounting media with dapi  
20 (Vectastain) was applied. Dapi stains nuclei and provides a counterstain.

## II. Results

TABLE 3  
Antibody Preparations and FACS Analysis

ANTIBODY	dye/protein (D/P) ratio	Post-labeling FACS analysis
2395 (6F8)	4	shift
2399 (8D11)	4	shift
2403 (5F2)	3	shift
Herceptin (4D5)	5	shift
anti-gd (952)	5	shift

25 To confirm that Cy3 labeling of the antibodies did not negatively affect their ability to bind antigen, the antibodies were tested for their binding specificity on cells by FACS analysis. In Table 3 "shift" refers to a shift in the cell population when screened for fluorescence and indicates that the Cy3 labeled antibodies maintain their abilities to bind their specific antigen.

TABLE 4  
Summary of Antibody Localization

ANTIBODY	MCF-7Her2gdPSCA Tumors staining positive	MCF-7 Tumors staining positive
2395 (6F8)	3 (n=4)	0 (n=3)
2399 (8D11)	2 (n=2)	0 (n=2)
2403 (5F2)	3 (n=4)	0 (n=3)
Herceptin(4D5)	2 (n=2)	0 (n=2)
anti-gd(952)	2 (n=2)	0 (n=2)
ANTIBODY	HCT-gdPSCA Tumors staining positive	
2395 (6F8)	1 (n=1)	
2399 (8D11)	2 (n=2)	

The results summarized in Table 4, demonstrated that the anti-PSCA antibodies were able to specifically localize and bind to PSCA-expressing tumor cells in vivo. No staining was detected in the lung or the kidney. Cy3 staining was observed in the liver for all PSCA antibodies, anti-gd, and Herceptin in what are most likely phagocytic Kupffer cells that have taken up the antibody. Non-malignant cells associated with the tumors, most likely macrophages, were also seen to stain positive for Cy3 for all antibodies on both the transfected and non-transfected cell lines. Additionally, antibody 6B8 was also shown to localize to PSCA-expressing PC-3 tumor cells but not on control-transfected PC3 tumor cells in vivo. The staining pattern was primarily membranous. Positive staining was also observed in macrophages in and adjacent to the tumor.

### Example 3

This example describes the ability of the anti-PSCA monoclonal antibodies of the invention, to be internalized by PSCA-expressing cells. These studies were performed using fluorescent or radiolabeled antibodies or antibodies with gold adducts. With the radiolabeled antibodies, quantitation of the amount of antibody internalized in each cell line was analyzed.

#### I. Materials and Methods

##### *In vitro* studies

Transfected cells expressing gD PSCA (HCT-47 and PC3-19 cells) and MCF7cells expressing PSCA/HER2 (also referred to herein as MCF7/PSCA/ HER2 cells) were grown on coverslips, incubated with a series of monoclonal anti-PSCA antibodies (10 $\mu$ g/mL) for one hour (hr), washed in PBS, fixed in 3% PBS buffered formaldehyde, permeabilized with 1% Triton in PBS and labeled with Cy3-fluorescent labeled anti-mouse IgG. In control experiments, parental, untransfected cells were used. Immunolabeled cells were observed in a Molecular Dynamics confocal microscope.

For electron microscopy, transfected NIH-3T3 expressing gD-PSCA were incubated at 4°C with 25ug/ml of anti-gD antibody for one hr. In another set of experiments, CHO cells expressing PSCA were



incubated at 4°C with 20µg/ml of 10E3 and 6C3 anti-PSCA antibodies. Following incubation with primary antibodies, the cells were treated with 10 nm gold adducts of goat anti-mouse IgG for one hour. The cells were switched to 37°C for 15 min and 1 hr before fixation in Karnovsky's fixative, and processed for electron microscopy. Thin sections were observed in a Philips CM12 equipped with a digitizing GATAN camera.

5 For electron microscopy autoradiography, 10A1 and 8D11 anti-PSCA antibodies were iodinated according to the manufacturer's instructions for Iodo-Gen. <sup>125</sup>I-10A1 and <sup>125</sup>I-8D11 anti-PSCA antibodies were incubated for 15 min, 1hr and 6hr with HCT116 gD PSCA cells (human colon tumor cell line transfected with gD human PSCA, described above). After incubation, the cells were washed and fixed in 2% formaldehyde, 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2 and postfixed in 1% osmium tetroxide. After dehydration and embedding in EPONATE 12 epoxy resin, thin sections were cut, coated with Ilford L4 emulsion. After exposure, the sections were developed in Microdol X, stained with uranyl acetate and lead citrate, and observed in a Philips CM12 electron microscope.

Internalization of 6B8 anti-PSCA antibody was studied by electron microscopy using PC3 cells expressing gD.huPSCA. 6B8 antibodies were iodinated according to manufacturer's instructions for Iodo-Gen. <sup>125</sup>I-6B8 antibodies were incubated for 15 min and 24 hr with PC3.gD.huPSCA clone4 cells. After incubation, the cells were washed and fixed with 2% formaldehyde, 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2 and postfixed with 1% osmium tetroxide. After dehydration and embedding in EPONATE 12 epoxy resin, thin sections were cut and coated with Ilford 1.4 emulsion. After exposure, the sections were developed in Microdol X, stained with uranyl acetate and lead citrate, and observed in a Philips CM 12 electron microscope.

A steady state internalization study was performed as follows: MCF7/Her2 cells (Fig. 11, right panel) are transfected with vector alone containing the puromycin selectable marker (PurR); this cell line served as negative control for internalization of the PSCA antibodies. MCF7/Her2/gD.PSCA cell line is described in Example 2. MCF7/Her2 and MCF7/Her2/gD.PSCA cells were incubated with one of the following antibodies for 5 hours at 37°C: 22nM <sup>125</sup>I labeled anti-PSCA monoclonal antibodies 2395, 2399, 2403, and 0.8nM <sup>125</sup>I labeled anti-Her2 antibody, Herceptin. Unbound antibody was removed and the cells were washed with cold medium. The amount of radioactivity bound to the cells was determined by incubating the cells in Acid Wash (2M Urea, 0.5M NaCl, 50mM Glycine pH2.4) for 10 minutes and then removing and counting the wash. The cells were then solubilized with Lysis Buffer (8M Urea, 3M glacial acetic acid) and the resulting lysates counted to determine the amount of radioactivity internalized. Using the specific activity of the radio-labeled antibodies and the number of cells in each assay sample, cpm were converted to molecule/cell.

### ***In vivo studies***

35 MCF7/HER2/PSCA xenograft tumor bearing mice were dosed IP with Cy3 labeled 6F8, 5F2, or 8D11 PSCA antibodies. After 24 hrs, the tumors were removed and cryosectioned at 5, 10, 20 and 50µm and observed by confocal microscopy.

Anti-PSCA antibodies are labeled with <sup>125</sup>I as described for the *in vitro* experiment above. MCF-7/Her2/gdPSCA cells are prepared and inoculated into NCR nude mice and <sup>125</sup>I labeled anti-PSCA

antibodies, a <sup>125</sup>I-anti-gd antibody, or <sup>125</sup>I labeled HERCEPTIN® are injected into the mice as described in Example 2 above. Tumor cells are sectioned for analysis.

## II. Results

In the *in vitro* studies, confocal microscopy of immunolabeled HCT 47 gD PSCA, PC3-19 gD PSCA and MCF7/HER2/PSCA cells showed that these cells bind and internalize the following anti-PSCA monoclonal antibodies, 8D11, 10A1, 6C3, 6F8, 5F2, 7A6 and 10E3 to varying extents (See Figures 5-8). Internalization staining pattern was characterized by the presence of cytoplasmic vesicles and perinuclear organelles likely to correspond to the endosomes in proximity of the Golgi apparatus.

In the *in vivo* study, labeling can be seen on the cell surface of most of the cryosectioned cells and internalized into small vesicles near the cell surface in a small percentage of the cells.

EM internalization studies using the above-described 3T3 cells incubated with anti-gD antibodies visualized with 10 nm gold particles indicated that gD-PSCA was internalized and transported to the endosomal compartment. Gold particles were visualized in calveolae and multivesicular bodies. EM internalization studies using PSCA-transfected CHO cells also showed that the same pathway was involved in the internalization of both 10E3 and 6C3 antibodies (see Figure 9A-9D). The kinetics of PSCA internalization appeared to be rapid (15 min from the plasma membrane to the endosomes).

With <sup>125</sup>I-10A1 and <sup>125</sup>I-8D11 anti-PSCA antibodies, autoradiographic silver grains were observed associated with the cell membrane, particularly with the microvilli. Autoradiographic grains were also seen internalized within the cells as early as 15 minutes after addition of the <sup>125</sup>I-labeled 10A1 and 8D11 antibodies. The internalization seen in the micrograph of Figure 10 is representative of the results of this experiment. Control HCT116 cells transfected with neo marker but not PSCA, showed no internalization.

The results with the 6B8 antibody were similar to those obtained with <sup>125</sup>I-10A1 anti-PSCA antibody. Internalization of antibody 6B8 was observed by 15 minutes, continuing through the 24-hour time-point. Autoradiographic grains were first observed associated with the caveolae of the cell membrane and later internalized in the endosomes.

Figure 11 shows the steady state levels of internalization of three anti-PSCA antibodies [ascites no. 2395 (6F8), 2399 (8D11), 2403(5F2)] at 5 hours in Her2 -expressing MCF7 cells transfected with gD-PSCA. Herceptin served as a control antibody. The number of molecules of anti-PSCA antibodies per cell bound versus free in the lysate, was quantitated.

## III. Conclusion

The antibodies directed against PSCA (6C3, 10E3, 10A1, 6F8, 5F2, 8D11, 6B8) were efficiently endocytosed and internalized both *in vivo* and *in vitro*. Ultrastructural analysis indicated that the internalization of 6C3, 10E3 and 6B8 likely takes place via calveolae. The antibodies accumulate in multivesicular bodies in close proximity to the Golgi apparatus. Thus, by different approaches, the anti-PSCA antibodies of the invention were shown to be internalized upon binding to PSCA on the cell surface.

#### Example 4

The abundance of PSCA in various normal and tumor tissues was assessed by determining from a database of cDNA libraries, the number of copies of the PSCA nucleotide sequence present in cDNA libraries representing genes expressed in different tissues and organs (see Figure 14). For each organ indicated in Figure 18 (x-axis), pools of several dozen cDNA libraries are represented. "All" means a mixture of the cDNA libraries representative of the different organs indicated in the figure, as well as cDNA libraries prepared from various tissues including bone, uterus, small intestine, thymus, stomach, connective tissue and unclassified tissue type. The relative abundance in normal and tumor organs is determined by the number of copies of the PSCA sequence in the pooled library divided by the total number of sequences in that pooled library and is represented in Figure 14 as #hits/100,000 random sequences. From the absolute abundance (Absabu), it is clear that PSCA expression is highly upregulated in prostate and bladder tumors.

#### Example 5

The anti-tumor activity of the anti-PSCA antibodies was assessed in vivo.

##### I. Materials and Methods

The human prostate cancer cell line, PC3, was obtained from the ATCC (ATCC CRL-1435). The cell line PC3.gDhuPSCA subclone was obtained by transfecting the parental PC3 line with pRK.gDPSCA.tkneo using the Eugene method (Roche Molecular Biochemical) and plating in 400ug/ml G418 (Life Technologies). After two weeks of growth, G418 resistant sub-clones were selected, expanded and assayed for gDPSCA expression. The cell line PC3.Neo was made by transfecting the parental PC3 line with the vector pRK.tkneo and selection in 400ug/ml G418. Stable colonies were pooled and expanded.

NCR nude mice (Taconic, Germantown, New York, USA) were injected subcutaneously with  $5 \times 10^6$  gDPSCA transfected PC-3 cells or neo transfected PC-3 cells. Anti-PSCA monoclonal antibodies 2395 (6F8), 2399 (8D11), 2403 (5F2), and 2761 (6B8) were administered intraperitoneally by injection at a dose of 10 mg/kg one day prior to cell inoculation. As a negative control, an anti-ragweed monoclonal antibody, MARG2 (Genentech), was administered by the same dosing regimen. Following cell inoculation, animals were treated biweekly with 10 mg/kg antibody. Tumor measurements were taken biweekly. The results of these experiments are shown in Figures 18-20.

In a separate experiment (posttreatment), tumors were allowed to establish to a certain size before the mice were treated with anti-PSCA antibody, mimicking the condition and treatment in prostate cancer patients. Optimally, the tumor size should be approximately  $75\text{-}100\text{mm}^3$  before antibody treatment. In this experiment, the NCR nude mice (Taconic, Germantown, New York, USA) were injected subcutaneously with  $5 \times 10^6$  gDPSCA transfected PC-3 cells and tumors were allowed to grow for 1 week. Mice were then grouped into four groups of nine mice, each group with a mean tumor volume of approximately  $200\text{mm}^3$ . The mice were then treated biweekly with 10 mg/kg anti-PSCA monoclonal antibodies 2395 (6F8), 2399 (8D11), 2761 (6B8), and 2403 (5F2) or anti-ragweed, MARG2 (Genentech). Tumor measurements were taken biweekly. The results of these experiments are shown in Figure 21.

## II. Results

The four anti-PSCA antibodies tested, i.e., 2395 (6F8), 2399 (8D11), 2403 (5F2), and 2761 (6B8), showed cytotoxic activity as naked (unconjugated) antibodies in killing the PSCA-expressing prostate cancer cells in vivo (see Figures 19-21), compared to the control ragweed antibody. The p values were significantly different from the control group at  $p < 0.05$  for 2395 and 2399 in Fig. 19, 2399, 2403 and 2761 in Fig. 20, and 2395 in Fig. 21. Tumoricidal activity was specific to PSCA expressing tumor cells (Figures 19-21) and absent in the control, PSCA-negative PC3 tumor cells (Figure 18). It was difficult to predict or control the growth rate of the PC3 tumor cells once injected into the mice, to timely initiate the antibody treatment. As a result, in these initial posttreatment experiments, the tumors were at a bigger ( $150\text{-}200\text{ mm}^3$ ) than optimum size ( $75\text{-}100\text{ mm}^3$ ) at the time of administration of the anti-PSCA antibody. Greater efficacy in tumor cytotoxicity can be expected if treatment with anti-PSCA antibody is initiated at a time when the tumor is smaller and of a more representative size and if the antibody dose was increased above  $10\text{ mg/kg}$ .

### Example 6

#### Cynomolgus Monkey PSCA

This example describes the cloning of the Cynomolgus monkey PSCA and shows that the present anti-human PSCA antibodies bind the highly homologous Cynomolgus PSCA and human PSCA with similar affinities. In addition to the high homology, we have found that Cynomolgus urothelium expresses PSCA in a manner that is identical to that observed for human urothelium, thus making this primate a good and appropriate animal model for anti-PSCA antibody toxicity studies. Naked and toxin-conjugated anti-PSCA antibodies will be tested in this model.

## I. Materials and Methods

### Cloning of Cynomolgus PSCA

RNA was purified from one cynomolgus monkey bladder. To clone PSCA, RT-PCR was performed using primers psca.2F (AAGGCTGTGCTGCTTGCCCT - SEQ ID NO: 14) and psca.1R (GAGTGGCACAAAGGCCTGGG - SEQ ID NO: 15). The resulting PCR products from two independent PCR reactions were subcloned into the PCR subcloning vector pCR2.1-TOPO (Invitrogen) and then sequenced.

#### **Affinity Measurements on Cell Surface PSCA**

The entire coding sequence for human and for Cynomolgus PSCA were cloned into an expression vector, pRK. Anti-PSCA monoclonal antibody 5F2 (ascites no. 2403) was labeled with  $^{125}\text{I}$  using the lactoperoxidase method. PC3 cells or CHO cells were transiently transfected with either pRK.gD.human psca or pRK.gD.cynomolgus psca using Eugene Reagent (Roche). After 2 days of transfection, the cells were replated in a 24 well dish and incubated in medium with various concentrations of unlabeled Mab 2403 and a constant amount of  $^{125}\text{I}$ -Mab 2403 for 16 hours at  $4^\circ\text{C}$ . Unbound antibody was removed and cells were washed with ice-cold medium. The amount of radioactivity bound was determined by solubilizing the cells

in 8M Urea/3M glacial acetic acid. The Kd value was calculated by scatchard analysis using the Newl program.

#### FACS Analysis:

5 COS Cells (African green monkey kidney cells) were transiently transfected with either pRK.human.pscA or pRK.cynomolgus.pscA. Two days after transfection, cells were removed from the dishes with 5mM EDTA and washed 1X with PBS. Cells were divided into several tubes and stained for one hour with 10ug/ml antibody 8D11 (ascites #2399) or 6B8. After washing 2X with PBS, cell stained with FITC anti-mouse (ICN) antibodies and then analyzed using flow cytometry. Xeno  
10 antibodies (from 10C5 subclones 1D8, 3B8 and 6D1) were used at 100ul of hybridoma supernatant reaction and were stained with FITC anti-human Kappa chain antibodies and again analyzed by cytometry. The results of the FACS analysis are shown in Table 5 below.

## II. Results

15 Seven subclones of the monkey PSCA were sequenced from which two types of sequence obtained: type I (4 subclones) and type II (3 subclones). The DNA and translated amino acid sequence shown in Figure 15 for type I (SEQ ID NO. 16 & 17) and Figure 16 for type II (SEQ ID NO. 18 & 19). RNA was purified from an individual monkey bladder and the monkey was from the wild (not an strain), these two types most likely represent different alleles of the same gene. Type I and T  
20 Cynomolgus PSCA show 98.64% identity at the DNA level, and 99.18% identity at the protein level only a single amino acid difference the two, at amino acid 119 (Ser vs. Gly). PSCA is a GPI-anchored surface protein. In human PSCA, the cleavage position for GPI attachment is at amino acid 100. Assuming that cleavage and GPI-linkage occurs at a similar position in Cynomolgus PSCA, the mature type I and II monkey PSCA would be 100% identical in amino acid sequence. Both types show approximately  
25 identity to human PSCA across the entire coding sequence. Figure 17 compares the amino acid sequence of human (SEQ ID NO. 1) and Cynomolgus Type I PSCA (SEQ ID NO.17).

The mean FACS shifts (indicated in units of FITC) for anti-PSCA antibodies on human and Cynomolgus PSCA expressed in COS Cells are shown in Table 5 (for murine antibodies) and Table 6 (for Xenomouse antibodies). It was known from other studies that the 5F2, 8D11 and 6F8 antibodies have  
30 similar binding affinities for human PSCA. Therefore, of these 3 antibodies, 5F2 was used as representative in the cold competition assay. Table 7 compares the affinities of antibodies 5F2 and 6F8 for human and Cynomolgus PSCA as determined by cold competition assay. The results from COS and CHO transfected cells were similar. The results from FACS analysis and cold competition indicated that the murine and xenomouse human antibodies bound human and cynomolgus PSCA that  
35 expressed in its native context on the cell surface, with equivalent affinity (within the margin of error).

TABLE 5 - Mean FACS Shifts

	<b>no Mab</b>	<b>8D11</b>	<b>6B8</b>
human	4.2	22.6	11
cynomolgus	3.9	21.9	10

5

TABLE 6 - Mean FACS Shifts

	<b>no Mab</b>	<b>1D8</b>	<b>3B8</b>	<b>6D1</b>
human	3.6	10	9.6	14.3
cynomolgus	3.8	10.9	12.3	14.4

TABLE 7 - Antibody Affinity for Cell Surface PSCA

10

<b>anti-ps.ca</b>	<b>PSCA</b>		
<b>mab</b>	<b>species</b>	<b>cell line</b>	<b>K<sub>d</sub></b>
5F2	human	PC3.gD.hu.ps.ca clone 4	13.2
5F2	cynomolgus	PC3.cyn.ps.ca clone 8	7
6B8	human	PC3.gD.hu.ps.ca clone 4	21
6B8	cynomolgus	PC3.cyn.ps.ca clone 8	3.3

**Example 7****Anti-PSCA Antibody - DM1 Conjugation**

15 This example describes the conjugation of an anti-PSCA antibody to the maytansinoid, DM1. DM1 is a prodrug that gets activated in the cell; it is a potent inhibitor of microtubule formation and disrupts mitosis. The structure of DM1 is shown in Figure 22. The structure of anti-PSCA antibody-DM1 conjugates is illustrated in Figure 23. For the following conjugation, the R group in Figure 22 is SH or a protected derivative thereof; particularly preferred is a disulfide derivative. Modifications may be made to this  
20 protocol, e.g., to optimize the pH or concentrations of reagents for individual antibodies; these modifications are routine in nature and will be familiar to a protein chemist of skill in the art.

**Modification of anti-PSCA antibody with SPP**

25 A purified anti-PSCA antibody (e.g., one of the anti-PSCA antibodies listed in Table 2B) is modified with N-succinimidyl-4-(2-pyridylthio) pentanoate (SPP) to introduce dithiopyridyl groups. The antibody (e.g., at about 6-10 mg/mL) in potassium phosphate buffer (PPB; e.g., 50 mM PPB at pH 6.5)

containing NaCl (50 mM) and EDTA (1 mM) is treated with SPP (e.g. at 5.3 molar equivalents in 2.3 mL ethanol). After incubation for 90 minutes under argon at ambient temperature, the reaction mixture is filtered following standard procedures e.g., by gel filtration through a Sephadex G25 column equilibrated with 35 mM sodium citrate, 154 mM NaCl, 2 mM EDTA. Antibody containing fractions are pooled and assayed. The degree of modification of the antibody is determined as described above.

#### Conjugation of anti-PSCA antibody-SPP-Py with DM1

The modified antibody (e.g., with 9.5  $\mu$ mol of releasable 2-thiopyridine groups) is diluted with the above 35 mM sodium citrate buffer, pH 6.5, to a final concentration of e.g., 2.5 mg/mL. DM1 (e.g., at 1.7 equivalents, 16.1  $\mu$ mol) in 3.0 mM dimethylacetamide (DMA, 3% v/v in the final reaction mixture) is then added to the antibody solution. The reaction proceeded at ambient temperature under argon for 20 hours.

The reaction is loaded on a gel filtration column, e.g., Sephacryl S300 gel filtration column (5.0 cm x 90.0 cm, 1.77 L) equilibrated with 35 mM sodium citrate, 154 mM NaCl, pH 6.5. The flow rate is at e.g., 5.0 mL/min and 65 fractions (20.0 mL each) are collected. The major peak comprises monomeric anti-PSCA antibody-DM1. Fractions around the major peak are pooled and assayed. The number of DM1 drug molecules linked per antibody molecule is determined, e.g., by measuring the absorbance at 252 nm and 280 nm, and found to be around 3-5 drug molecules per antibody molecule.

#### Example 8

##### Cytotoxicity of anti-PSCA-DM1 conjugates on cultured cells

Anti-PSCA antibodies #2761 (6B8) and #2399 (8D11) were conjugated to DM1 as described above. From the internalization studies, it was expected that the DM1 antibody conjugates should be efficiently taken up by PSCA expressing cells. Two anti-ragweed antibodies were used as controls for antigen specificity and immunoglobulin isotype. The results for the *in vitro* cytotoxicity of the first four DM1 conjugates are reported. Cytotoxicity was measured in clonogenic assays on PC3.Neo cells and PC3.gD human PSCA clone 4 cells. These two cell lines were shown to be equally sensitive to the non-conjugated DM1 (Fig. 24), suggesting that PC3.Neo cell line was a good antigen-negative control for the PC3.gD human PSCA clone 4 cells.

#### I. Method

Cells were plated in 6-well tissue culture grade plates in growth medium that contained or did not contain the antibody conjugate to be tested. Control cultures were plated at 500 cells/plate. Cultures that were exposed to the conjugate were plated at several densities between 500 and 5000 cells/well. The cells were then incubated at 37°C for 5 to 7 days to allow colony formation. The cells were fixed, stained with Crystal violet dye, and the colonies were scored. Plating efficiency (PE) was calculated as the number of colonies/number of cells plated. Surviving fraction was calculated as PE of treated cells/PE of non-treated cells. Plating efficiencies of control cultures were in the range of 40 to 60% for both the control and the PSCA-expressing cell line.

## II. Results: (concentrations are given as those for the respective antibodies)

Conjugate	Reference #	Isotype	DM1/Ab	IC <sub>10</sub> , M
Anti-PSCA antibody #2761-DM1	1461-44	IgG <sub>2a</sub>	3.39	$\sim 1 \times 10^{-9}$
5 Anti-PSCA antibody #2399-DM1	1461-165	IgG <sub>2b</sub>	2.94	$\sim 3 \times 10^{-10}$
Anti-Ragweed antibody #1428-DM1	1461-154	IgG <sub>2a</sub>	2.68	not toxic
Anti-Ragweed antibody #1429-DM1	1461-175	IgG <sub>2b</sub>	1.85	not toxic

Antibody conjugate 2399-DM1 was cytotoxic for PC3.gDhuman PSCA clone 4 cells with an  
 10 IC<sub>10</sub>  $\sim 3 \times 10^{-10}$  M, continuous exposure (Fig. 25). An isotype-matched control, 1429-DM1 was not toxic for  
 the cells up to the highest concentration tested ( $3 \times 10^{-9}$  M). The second conjugate, 2761-DM1, was  
 cytotoxic for PC3.gDhuman PSCA clone 4 cells with an IC<sub>10</sub>  $\sim 1 \times 10^{-9}$  M, continuous exposure (Fig. 26).  
 An isotype-matched control, 1428-DM1 was not toxic for the cells up to the highest concentration tested ( $3 \times$   
 15  $10^{-9}$  M). None of the four antibody-DM1 conjugates were toxic for PC3.Neo cells up to the highest  
 concentration tested ( $3 \times 10^{-9}$  M), see Fig. 27.

### Conclusion

Antibody conjugates 2399-DM1 and 2761-DM1 were specifically cytotoxic towards the PSCA  
 antigen-positive cells.

## 20 Example 9

### Effect of Antibody-DM1 Conjugates on Tumor Growth in Vivo

#### I. Materials and Methods

Female NCR nude mice (Taconic, Inc.) 6-8 weeks of age were injected with  $5 \times 10^6$  PC-3gdPSCA  
 cells subcutaneously in a volume of 0.2 ml on Day 1. Tumors were allowed to grow for 5 days and then were  
 25 measured in two dimensions using a caliper. Tumor volume was expressed in mm<sup>3</sup> using the formula:  $V =$   
 $0.5a \times b^2$ , where a and b are the long and the short diameters of the tumor, respectively. Mice were grouped  
 into 6 groups of 7 mice with a mean tumor volume of 160 mm<sup>3</sup>. On Day 6, antibody treatments were started.  
 Naked (non-DM1 conjugated) anti-PSCA antibodies 2399 (8D11), 2761 (6B8) and the anti-Ragweed  
 negative control antibody, 1428, were injected intraperitoneally (IP) twice a week at a dose of 10 mg/kg. This  
 30 treatment regimen was continued through out the experiment. The same antibodies conjugated to the  
 maytansinoid cytotoxin, DM1, were injected intravenously (IV) at a concentration of 75 ug/kg of DM1.  
 DM1 antibodies were administered twice a week for a total of 8 doses. Tumors were measured twice a week  
 through out the experiment. Table 8 below shows the dosing regimen.

TABLE 8

Group	Antibody	DM1 dose	AB dose	Schedule
1	2399-naked IP	N/A	10 mg/kg	2X week entire duration
2	2761-naked IP	N/A	10 mg/kg	2X week entire duration
3	Anti-Ragweed Naked (1428)	N/A	10 mg/kg	2X week



	IP			entire duration
4	2399-DM1 IV	75 ug/kg	5.5 mg/kg	2X week 8 total doses
5	2761-DM1 IV	75 ug/kg	5 mg/kg	2X week 8 total doses
6	Anti-ragweed-DM1(1428) IV	75 ug/kg	6.1 mg/kg	2 X week 8 total doses

## Results

As shown in Figure 28, by day 26 of treatment, the human prostate tumors (PSCA-expressing cells) were virtually eliminated in the animals treated with DM1-conjugated anti-PSCA antibodies (2399 and 2761) compared to both the non-conjugated anti-PSCA antibodies and the DM1-antiragweed control antibody. Within each group of 7 animals treated with DM1-2399 or DM1-2761, four of the animals had no detectable tumor. For the three animals with remaining tumor, the average tumor size was just 6 mm<sup>3</sup> compared to a tumor volume of about 550 mm<sup>3</sup> for the DM1-ragweed control antibody group. DM1 treatments will continue for one more dose ending on day 29. On day 33, mice from all groups are sacrificed and tumor tissues are collected for histological analysis. Other tissues including liver, kidney and bladder are collected and examined for evidence of cytotoxicity related to antibody treatments.

## Example 10

### PSCA Expression Analysis Using Tissue Microarray

The expression of human PSCA in normal tissues and malignant tumors was investigated using isotopic in-situ hybridization (ISH) on multi-tissue arrays. A tissue microarray (TMA) is a paraffin block which typically contains between one hundred to more than one thousand individual tissue samples. TMAs are constructed by taking small biopsy samples from "donor" tissues embedded in paraffin blocks and re-embedding the biopsies together in a single "recipient" block to form an array.

#### I. Method

PCR primers (upper- 5' ACC CAC GCG TCC GGC TGC TT 3' [SEQ ID NO. 24] and lower- 5' CGG GGG ACA CCA CGG ACC AGA 3' [SEQ ID NO. 25]) were designed to amplify a 768 bp fragment of human PSCA. Primers included extensions encoding 27-nucleotide T7 or T3 RNA polymerase initiation sites to allow in vitro transcription of sense or antisense probes, respectively, from the amplified products. Tumor microarray slides were deparaffinized, deproteinized in 20 µg/ml of proteinase K for 15 minutes at 37 °C, and further processed for *in situ* hybridization. <sup>33</sup>P-UTP labeled sense and antisense probes were hybridized to the sections at 55°C overnight. Unbound probe was removed by incubation in 20mg/ml RNase A for 30 min at 37°C, followed by a high stringency wash at 55°C in 0.1 X SSC for 2 hours and dehydration through graded concentrations of ethanol. The slides were dipped in NBT2 nuclear track emulsion (Eastman Kodak), exposed in sealed plastic slide boxes containing desiccant for 4 weeks at 4°C, developed and counterstained with hematoxylin and eosin.

#### II. Results

The results of the ISH assay for normal prostate and prostate tumors are as summarized in Table 9.

TABLE 9

Tissue	In-situ Hybridization		
	Negative	Weakly Positive	Strongly Positive
Normal Prostate (n=29)	55%	28%	17%
Normal Urothelium (n=4)	0%	25%	75%
Primary Prostate Cancer (n=97)	48%	27%	25%
Metastatic Prostate Cancer (n=28)	22%	39%	39%

The group of primary prostate cancer contains seven cases of prostatic intraepithelial neoplasia (PIN; one case is negative, one case is weakly positive and five cases are strongly positive for PSCA). The group of metastatic prostate cancers consists of 25 cases of lymph node metastases, two cases of liver metastases (both cases are strongly positive for PSCA) and one case of a brain metastasis (weakly positive for PSCA). Preliminary results on an additional prostate multi-tissue array (normal prostate, primary and metastatic prostate cancer) confirm these numbers. The results for other tumor types (lung and colon) are preliminary and appear to indicate that approximately 10% of lung tumors and 5% of colon tumors express PSCA at a weak or moderate level.

#### Example 11

##### PSCA distribution in tumors and normal tissue

Example 11 describes the distribution of PSCA in tumors and normal tissue as evaluated by immunohistochemistry (IHC) using the anti-PSCA mAbs herein. A series of primary, organ-confined prostate cancers is evaluated for expression of PSCA. The expression of PSCA is also assayed in frozen sections of breast, lung, colon and kidney tumors, and in colon carcinoma cell lines. Separately, Taqman<sup>TM</sup> analysis for PSCA is being performed on some of the same cases used for this IHC study. TaqMan<sup>TM</sup> is a fluorescent PCR-based technique which makes use of the 5' exonuclease activity of Taq DNA polymerase enzyme to monitor gene amplification in real time.

##### **Staining Procedure for PSCA on Frozen Sections**

Five micron thick frozen sections are cut from fresh frozen tumor tissue on a Leica cryostat and stored at -20°C until further use.

Sections are dried thoroughly and fixed in Acetone/Ethanol for 5 minutes. They are then rinsed twice with phosphate-buffered saline (PBS), for 5 minutes each. Endogenous peroxidase activity is quenched using the glucose oxidase/glucose method at 37°C for 60 minutes. This is followed by rinses with PBS, 2 changes for 5 minutes each. Blocking for endogenous biotin is done using Vector Avidin/Biotin Blocking Kit. Blocking for endogenous immunoglobulin binding sites is done with 10% normal horse serum for 30 minutes. Blocking serum is not washed off.

The sections are incubated with anti-PSCA murine antibody at 10ug/ml diluted in blocking serum for 60 minutes at room temperature (RT). A mouse isotype control antibody (Zymed) is used for negative control. The sections are rinsed with PBS twice for 5 minutes each. The detection of specifically bound primary antibody is accomplished with biotinylated horse anti-mouse Ig. The sections are incubated with diluted biotinylated horse anti-mouse IgG 1:200 in blocking serum for 30 minutes. The sections are rinsed with PBS twice for 5 minutes each, incubated with diluted ABC reagent for 30 minutes and again rinsed with PBS twice for 5 minutes each. Sections are incubated with Pierce Metal Enhanced DAB for 5 minutes, then

rinsed with tap water for 5 minutes, counterstained with Mayer's hematoxylin for 1 minute, rinsed with water, transferred into 70%, 95% and 100% Ethanol, followed by xylene and coverslipped. The sections are then dehydrated, cleared and mounted in synthetic mounting media.

## 5 Results

PSCA expression in malignant epithelial cells is seen. The level of expression ranges from weak to very strong, the proportion of malignant cells positive ranges from less than 10% to >95%. PSCA mRNA has previously been demonstrated in a case of colorectal adenocarcinoma by in-situ hybridization.

## 10 Example 12

Animal models are useful for understanding pathogenetic mechanisms and for the development of novel therapeutics. This study reports the pattern of expression of the murine PSCA in fetal and adult tissues by *in situ* hybridization, as well as a murine model for human prostate adenocarcinoma.

Structurally, PSCA is a GPI-linked membrane glycoprotein with homology to members of the Thy-1/Ly-6 family of proteins. It is most closely related to Sca2, a cell surface maker for immature lymphocytes (Noda S. et al. Journal of Experimental Medicine 1996; 183:2355-60). Expression of human PSCA RNA in normal tissues was originally described not only as tissue specific, but essentially limited to prostatic basal cells. The authors inferred from this latter observation and from the structural homology to Sca2 that PSCA may represent a "stem" cell antigen.

The murine homologue has been shown to demonstrate 70% identity to human PSCA at the nucleotide and the amino acid level. (Reiter RE et al., 1998, Proc. Nat. Acad. Sci. 95:1735-40). One well established animal model for human prostate cancer is the Transgenic Adenocarcinoma of the Mouse Prostate™ (TRAMP™) (Greenberg NM, 1995, PNAS 92:3439-43). Though there are significant anatomical differences in the mouse prostate compared to that of the human, there are inherent common characteristics including secretory function and hormonal regulation. In the TRAMP™ model, the minimal promoter of the rat probasin gene targets expression of the SV40 large T- antigen to the prostatic epithelium. All male TRAMP mice progress to prostate cancer usually by 8-12 weeks (Gingrich JR et al., 1997, Cancer Research 57:4687-91). The autochthonous TRAMP™ model is particularly relevant to human prostate carcinoma in that the development of the cancer is specific to the prostatic epithelium and is initially regulated by androgens. Furthermore, metastases to distant sites eventually occur (Gingrich JR et al., 1996, Cancer Research 56:4096-102; Gingrich JR et al., 1999, Prostate Cancer & Prostatic Diseases 2:70-75).

## I. Materials and methods

### Mice and Tissue Collection

Male and female adult CD-1 mice between the ages of 10 and 24 weeks were obtained from Charles River laboratories. Pregnant mice were ordered for the collection of embryos. The day of isolation with copulatory plug was considered day 1 of embryonic development. Fetal tissues examined by ISH included: E10, E13, E14, E15, E17 and E18. Adult tissues examined included: tissues of the male urogenital system, female urinary bladder and kidney, pubescent mammary gland, 14 day pregnant mammary gland, lactating mammary gland, liver, heart, skin and intestine. All tissues were fixed in 4% formalin and paraffin-

embedded. Breeder pairs of TRAMP<sup>TM</sup> transgenic mice were obtained from Dr. Norman Greenberg (Baylor College of Medicine, Houston, TX). Urogenital tissues from wild type litter mate C57BL/6 were taken at age 12 and 24 for comparison to age matched TRAMP<sup>TM</sup> transgenic mice. By the age of 12 weeks, TRAMP<sup>TM</sup> mice have typically progressed to PIN and or well differentiated tumors. Nine TRAMP<sup>TM</sup> male mice between the ages of 12 to 39 weeks of age were sacrificed and tissues were collected for routine histology and ISH studies. The histological grade of prostate cancer was determined in according to previously published studies in the TRAMP<sup>TM</sup> model (Gingrich JR et al., 1999, Prostate Cancer & Prostatic Diseases 2:70-75).

#### Cloning of the Murine PSCA Orthologue

Full-length cDNA for murine PSCA was amplified from mouse 17-day embryo Marathon-Ready cDNA (Clontech). Primers were designed based on EST sequences present in Genbank. Sense primer (5' ACT ATG AAG CTT TGC AGC TCA TCC CTT CAC AAT CG 3' (SEQ ID NO. 20)) and anti-sense primer in the 3' untranslated region (5' GAA TTC GGA TCC ACC ATG AAG ACC GTC TTC TTT CTC CTG CTG 3' (SEQ ID NO. 21)) were used resulting in a 420 bp fragment that was subsequently cloned into the PCR subcloning vector PCR2.1TOPO (Invitrogen). Clones were confirmed by DNA sequencing.

#### In Situ Hybridization

PCR primers (upper- 5' CCT GCT GGC CAC CTA CT 3' and lower- 5' CCT TCA CAA TCG GGC TAT 3' - SEQ ID NOs. 22 & 23, respectively) were designed to amplify a 388 bp fragment of murine PSCA. Primers included extensions encoding 27-nucleotide T7 or T3 RNA polymerase initiation sites to allow in vitro transcription of sense or antisense probes, respectively, from the amplified products (Lu et al. Cell Vision 1994, 1:169-176). Five µm thick sections were deparaffinized, deproteinized in 4 µg/ml of proteinase K for 30 minutes at 37 °C, and further processed for *in situ* hybridization. <sup>33</sup>P-UTP labeled sense and antisense probes were hybridized to the sections at 55°C overnight. Unbound probe was removed by incubation in 20 mg/ml RNase A for 30 minutes at 37 °C, followed by a high stringency wash at 55 °C in 0.1 X SSC for 2 hours and dehydration through graded concentrations of ethanol. The slides were dipped in NBT2 nuclear track emulsion (Eastman Kodak), exposed in sealed plastic slide boxes containing desiccant for 4 weeks at 4°C, developed and counterstained with hematoxylin and eosin. The *in situ* hybridization was routinely performed on duplicate sections with sense and antisense probes. No significant hybridization signal was observed on sections hybridized with the sense probe.

#### Results

In summary of the results, murine PSCA is expressed during fetal development in the urogenital sinus, skin and gastrointestinal tract. The expression in these tissues is restricted to the most superficial cell layer. In the adult mouse, expression is highest in the mucosal lining of the urinary tract. In the normal adult prostate, expression of PSCA is detected exclusively in the secretory epithelium. Examination of PSCA during carcinogenesis of the murine prostate in the TRAMP<sup>TM</sup> model showed a markedly increased expression in areas of neoplasia.

These studies demonstrate that expression of murine PSCA is neither prostate specific or consistent with it being a stem cell marker. The results clearly indicate that PSCA expression in epithelial surfaces is

restricted to the adluminal or superficial cell layer and not, as reported for the human gene, to basal cells. Cells of the adluminal outer layer typically display a high degree of differentiation, but unlike stem cells, low proliferative or self-renewal capability. The designation of this molecule as a "stem cell antigen" is therefore questionable. These results suggest that the pattern of expression of murine PSCA in normal tissues and in tissues of murine prostatic adenocarcinoma is different from published data on human prostate adenocarcinomas. Most likely, the differences in PSCA expression between the TRAMP<sup>TM</sup> tumors and human prostatic adenocarcinoma either indicate species-specific regulation of PSCA expression or are related to the type of carcinogenic event at play.

The strongest expression of PSCA is detected in the urothelium of the developing and mature urinary tract. In the adult organism the expression appears continuous from the renal pelvis throughout the urethra. Prostatic expression is patchy, but increases noticeably at the juncture of the large prostatic ducts and urethra. It is tempting to speculate, whether this finding is related to urinary reflux into these structures providing a potential clue to the function of this protein. A common feature of the epithelial surfaces exhibiting PSCA expression is the fact that they line structures which are in continuous contact with fluids, mucous or secretions. This is obviously the case for urothelium, however, this notion also holds true for the observation that significant PSCA expression is present in the amniotic membrane, anal canal, oropharynx, esophagus and skin of the developing fetus. Not to be limited by any one hypothesis or mechanism, it is likely that additional denominators are at play, because other fluid-exposed epithelial tissues such as the fetal tracheobronchial tree, do not express PSCA. Whether the expression in fetal skin is temporally regulated is difficult to determine at this point as the number of embryos evaluated is small. However it appears that expression of PSCA in skin is no longer required in postnatal, extrauterine life. The pattern of expression within multilayered epithelial surfaces suggests that PSCA may function as a barrier or buffer against external insults. Further studies, such as the generation of PSCA knockout mice would be helpful to clarify the primary function of PSCA in normal tissue.

The rather selective expression of PSCA in fetal and adult tissues makes the mouse a suitable model to determine the physiologic function of this protein. Though PSCA is lost in the advanced tumor stages, the TRAMP<sup>TM</sup> model may still represent a potentially useful tool to study PSCA as a target for prostate cancer.

## CONCLUSION

The working examples above demonstrated that the anti-PSCA antibodies of the invention effectively targeted PSCA-expressing cancer cells *in vivo* and that the GPI-linked PSCA molecule and the antibody bound to it were rapidly internalized upon the binding of the antibody to a PSCA-expressing cancer cell. The anti-PSCA antibodies showed specificity and efficacy in tumor cell killing and arresting tumor growth *in vivo*. Cytotoxicity of the tumor cells was greatly enhanced using toxin-conjugated antibody, specifically the maytansinoid, DM1, which is a toxin that acts intracellularly. Treatment with DM1-conjugated anti-PSCA antibody completely eliminated the PSCA-expressing, human prostate tumor cells *in vivo* (see Example 9).

## IX. References

References cited within this application, including patents, published applications and other publications, are hereby incorporated by reference.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology and the like, which are within the skill of the art. Such techniques are explained fully in the literature. See *e.g.*, Molecular Cloning: A Laboratory Manual, (J. Sambrook *et al.*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989); Current Protocols in Molecular Biology (F. Ausubel *et al.*, eds., 1987 updated); Essential Molecular Biology (T. Brown ed., IRL Press 1991); Gene Expression Technology (Goeddel ed., Academic Press 1991); Methods for Cloning and Analysis of Eukaryotic Genes (A. Bothwell *et al.* eds., Bartlett Publ. 1990); Gene Transfer and Expression (M. Kriegler, Stockton Press 1990); Recombinant DNA Methodology II (R. Wu *et al.* eds., Academic Press 1995); PCR: A Practical Approach (M. McPherson *et al.*, IRL Press at Oxford University Press 1991); Oligonucleotide Synthesis (M. Gait ed., 1984); Cell Culture for Biochemists (R. Adams ed., Elsevier Science Publishers 1990); Gene Transfer Vectors for Mammalian Cells (J. Miller & M. Calos eds., 1987); Mammalian Cell Biotechnology (M. Butler ed., 1991); Animal Cell Culture (J. Pollard *et al.* eds., Humana Press 1990); Culture of Animal Cells, 2<sup>nd</sup> Ed. (R. Freshney *et al.* eds., Alan R. Liss 1987); Flow Cytometry and Sorting (M. Melamed *et al.* eds., Wiley-Liss 1990); the series Methods in Enzymology (Academic Press, Inc.); Wirth M. and Hauser H. (1993); Immunochemistry in Practice, 3rd edition, A. Johnstone & R. Thorpe, Blackwell Science, Cambridge, MA, 1996; Techniques in Immunocytochemistry, (G. Bullock & P. Petrusz eds., Academic Press 1982, 1983, 1985, 1989); Handbook of Experimental Immunology, (D. Weir & C. Blackwell, eds.); Current Protocols in Immunology (J. Coligan *et al.* eds. 1991); Immunoassay (E. P. Diamandis & T.K. Christopoulos, eds., Academic Press, Inc., 1996); Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; Ed Harlow and David Lane, Antibodies A laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988; Antibody Engineering, 2<sup>nd</sup> edition (C. Borrebaeck, ed., Oxford University Press, 1995); and the series Annual Review of Immunology; the series Advances in Immunology.

#### 25 X. Deposit of Cell Lines and DNA

The following hybridoma cell lines were deposited with the American Type Culture Collection (ATCC), located at 10801 University Boulevard, Manassas, Virginia 20110-2209, U.S.A., and accorded the accession numbers:

	<u>Hybridoma</u>	<u>ATCC Accession No.</u>	<u>Deposit Date</u>
30	PSCA.10E3	PTA-717	September 16, 1999
	PSCA.6F8	PTA-718	September 16, 1999
	PSCA.8D11	PTA-719	September 16, 1999
	PSCA.5F2	PTA-720	September 16, 1999
	PSCA.6C3	PTA-880	October 26, 1999
35	PSCA.6B8	PTA-2265	July 25, 2000
	PSCA.10C5	PTA-2264	July 25, 2000
The following vector DNAs (see Example 1) were deposited with the ATCC			
	pRK-2403L		October __, 2000
	pRK-2716L		October __, 2000
40	pRK-2403H		October __, 2000
	pRK-2716H		October __, 2000

The names of the deposited hybridoma cell lines above have been shortened for convenience of reference; these hybridomas correspond to the clones (with their full names) listed in Table 2B.

5 This deposit was made under the provisions of the Budapest Treaty on the International Recognition  
of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder  
(Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of deposit. The  
organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an  
agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the  
progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the  
10 public of any U.S. or foreign patent application, whichever comes first, and assures availability of the  
progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto  
according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with  
particular reference to 886 OG 638).

15 The assignee of the present application has agreed that if the cultures on deposit should die or be lost  
or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a  
viable specimen of the same culture. Availability of the deposited strains are not to be construed as a license  
to practice the invention in contravention of the rights granted under the authority of any government in  
accordance with its patent laws. The making of these deposits is by no means an admission that deposits are  
required to enable the invention.

20

WHAT IS CLAIMED IS:

1. An isolated anti-prostate stem cell antigen (PSCA) antibody that internalizes upon binding to PSCA on a mammalian cell in vivo.
- 5 2. The antibody of claim 1 which is a monoclonal antibody.
3. The antibody of claim 1 which is an antibody fragment.
4. The antibody of claim 1 which is a chimeric or a humanized antibody.
5. The antibody of claim 2 which is produced by a hybridoma selected from the group of hybridomas deposited under American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, or PTA-2264, or which has the amino acid sequence of SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, or SEQ ID NO.13.
- 10 6. The antibody of claim 2, wherein the antibody competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma selected from the group of hybridomas deposited under the American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, PTA-2264.
- 15 7. The antibody of claim 2 which is conjugated to a growth inhibitory agent.
8. The antibody of claim 2 which is conjugated to a cytotoxic agent.
9. The antibody of claim 8 wherein the cytotoxic agent is selected from the group consisting of toxins, antibiotics, radioactive isotopes and nucleolytic enzymes.
- 20 10. The antibody of claim 9 wherein the cytotoxic agent is a toxin.
11. The antibody of claim 10, wherein the toxin is selected from the group consisting of maytansinoid or calicheamicin
12. The antibody of claim 11, wherein the toxin is a maytansinoid.
13. The antibody of claim 12, wherein the maytansinoid has the structure shown in FIG. 22.
- 25 14. The antibody of claim 2, wherein the mammalian cell is a cancer cell.
15. An anti-PSCA monoclonal antibody that inhibits the growth of PSCA-expressing cancer cells in vivo.
16. The antibody of claim 15, wherein the antibody internalizes upon binding to PSCA on the cancer cell.
- 30 17. The antibody of claim 16 which is a humanized or human antibody.
18. The antibody of claim 17 which is produced in bacteria.
19. The antibody of claim 15, which is a humanized form of an anti-PSCA antibody produced by a hybridoma selected from the group of hybridomas having ATCC accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, or PTA-2265.
- 35 20. The antibody of claim 15, wherein the cancer cells are from a cancer selected from the group consisting of prostate cancer, bladder cancer and lung cancer.
21. The antibody of claim 20, wherein the cancer is prostate cancer.
22. An isolated nucleic acid comprising a sequence that encodes a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO.3, SEQ ID NO.4, SEQ ID NO.5, SEQ ID NO.6, SEQ ID NO.7, SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, or SEQ ID NO.13.
- 40



23. An expression vector comprising the nucleic acid of claim 22 operably linked to an expression regulatory sequence.
24. A cell that produces the antibody of claim 2.
25. The cell of claim 24, wherein the cell is selected from the group of hybridoma cells deposited  
5 under American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2264, or PTA-2265.
26. A host cell comprising the vector of claim 23.
27. The host cell of claim 26 which is a bacterial cell.
28. A method of producing the antibody of claim 2 comprising culturing the cell of claim 24  
10 and recovering the antibody from the cell culture.
29. A composition comprising the antibody of claim 2 or claim 15, and a carrier.
30. The composition of claim 29, wherein the antibody is conjugated to a cytotoxic agent.
31. The composition of claim 30, wherein the cytotoxic agent is a maytansinoid.
32. The composition of claim 31, wherein the antibody is a human or humanized antibody and  
15 the carrier is a pharmaceutical carrier.
33. The composition of claim 32, wherein the humanized antibody is a humanized form of an anti-PSCA antibody produced by a hybridoma selected from the group of hybridomas having ATCC accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, or PTA-2265.
34. A method of killing a PSCA-expressing cancer cell, comprising contacting the cancer cell  
20 with the antibody of claim 1, thereby killing the cancer cell.
35. The method of claim 34, wherein the cancer cell is selected from the group consisting of prostate cancer, bladder cancer and lung cancer cell.
36. The method of claim 35, wherein the cancer cell is a prostate cancer cell.
37. The method of claim 36, wherein the prostate cancer is androgen independent.
- 25 38. The method of claim 36, wherein the cancer cell is from metastatic prostate cancer.
39. The method of claim 34, wherein the antibody is an antibody fragment.
40. The method of claim 34, wherein the antibody is a humanized antibody.
41. The method of claim 40, wherein the antibody is conjugated to a cytotoxic agent.
42. The method of claim 41, wherein the cytotoxic agent is a toxin selected from the group  
30 consisting of maytansinoid or calicheamicin.
43. The method of claim 42, wherein the cytotoxic agent is a maytansinoid.
44. The method of claim 43, wherein the antibody is a humanized form of the antibody produced by a hybridoma selected from the group of hybridomas having ATCC accession number PTA-718, PTA-719, PTA-720, PTA-880, or PTA-2265.
- 35 45. The method of claim 41, wherein the cytotoxic agent is a radioactive isotope.
46. A method of alleviating a PSCA-expressing cancer in a mammal, comprising administering a therapeutically effective amount of the antibody of claim 15 to the mammal.
47. The method of claim 46, wherein the cancer is selected from the group consisting of prostate cancer, bladder cancer and lung cancer.
- 40 48. The method of claim 47, wherein the cancer is a prostate cancer.

49. The method of claim 48 wherein the prostate cancer is androgen independent or metastatic prostate cancer.
50. The method of claim 46, wherein the antibody is a humanized antibody.
51. The method of claim 50, wherein the antibody is conjugated to a cytotoxic agent.
- 5 52. The method of claim 51, wherein the cytotoxic agent is a maytansinoid.
53. The method of claim 52, wherein the maytansinoid has the structure shown in FIG. 22.
54. The method of claim 46, wherein the antibody is administered in conjunction with at least one chemotherapeutic agent.
55. The method of claim 54, wherein the chemotherapeutic agent is paclitaxel or derivatives thereof.
- 10 56. An article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody of claim 2, and further comprising a package insert indicating that the composition can be used to treat prostate cancer.

15

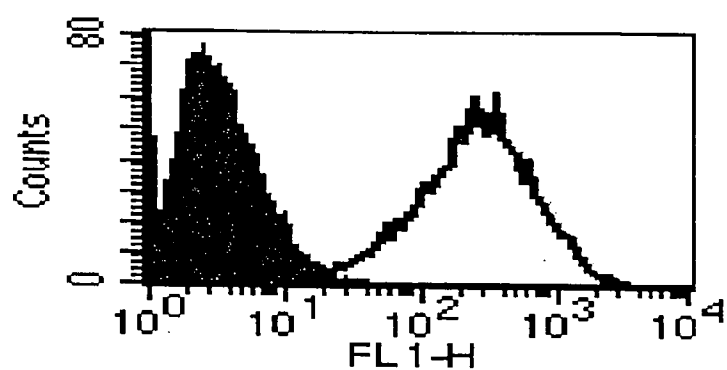


FIG. 1

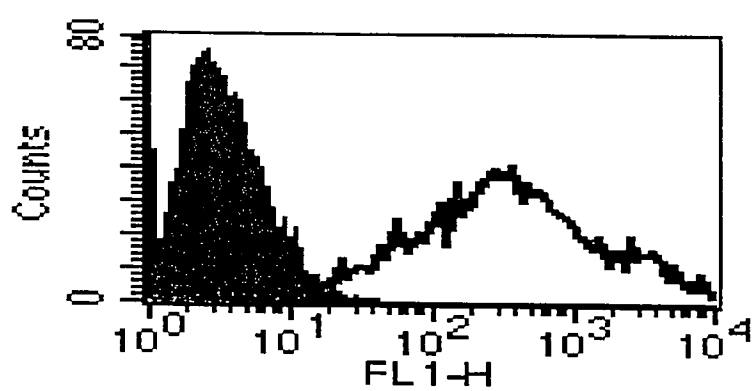


FIG. 2

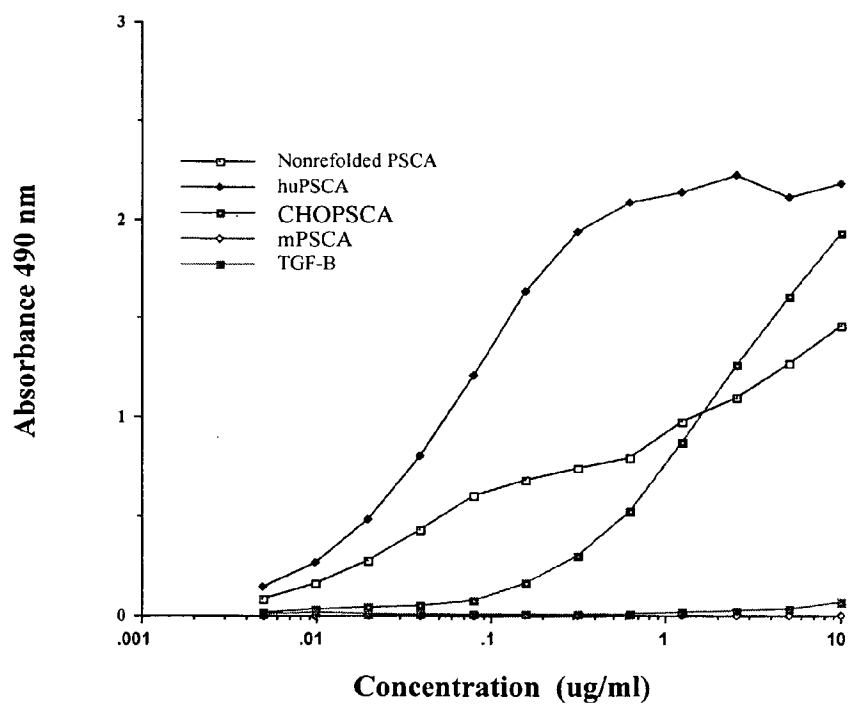


FIG. 3

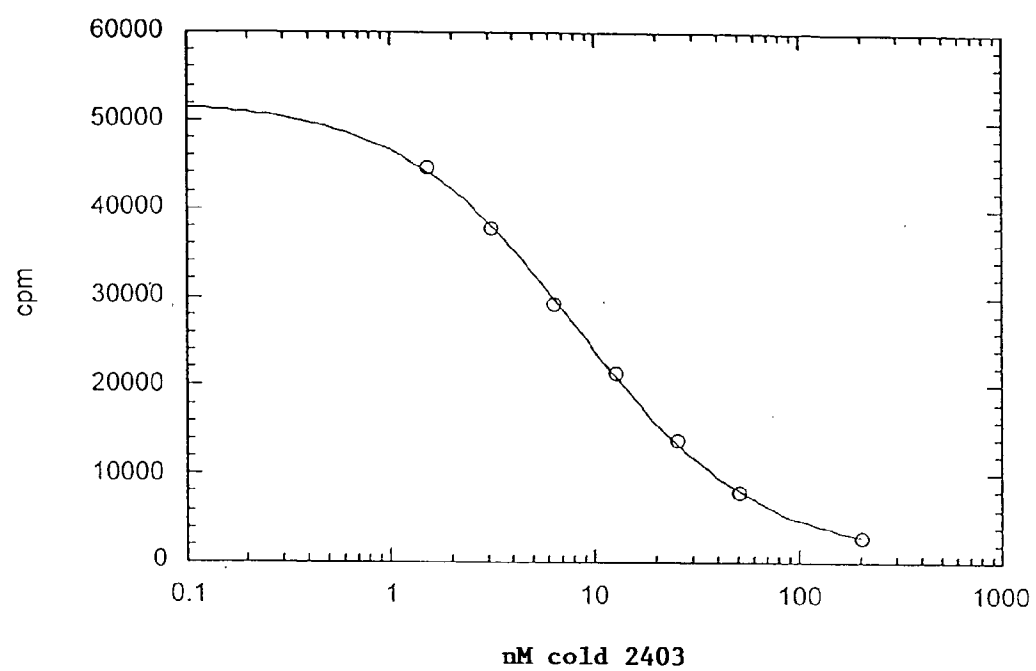


FIG. 4

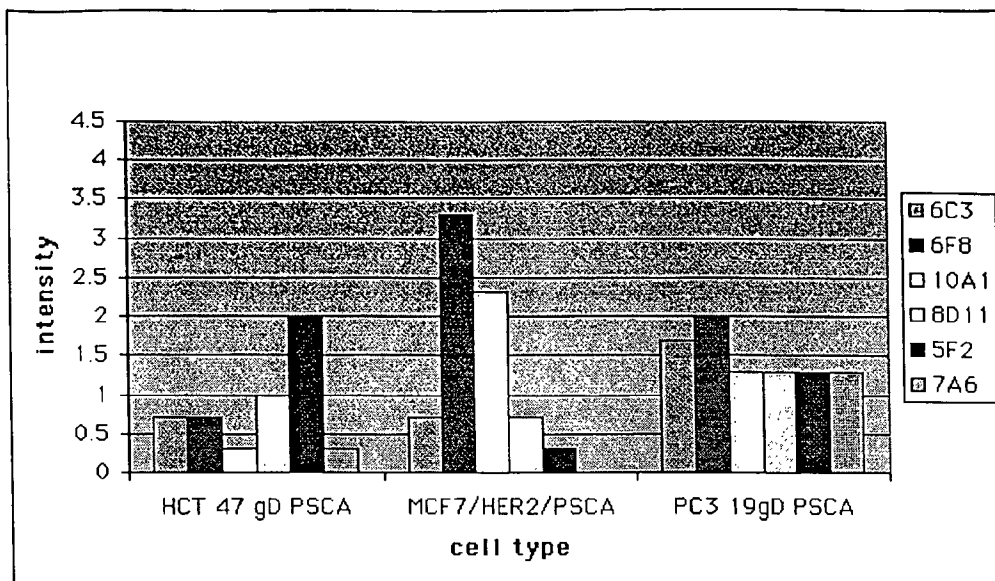


Fig. 5

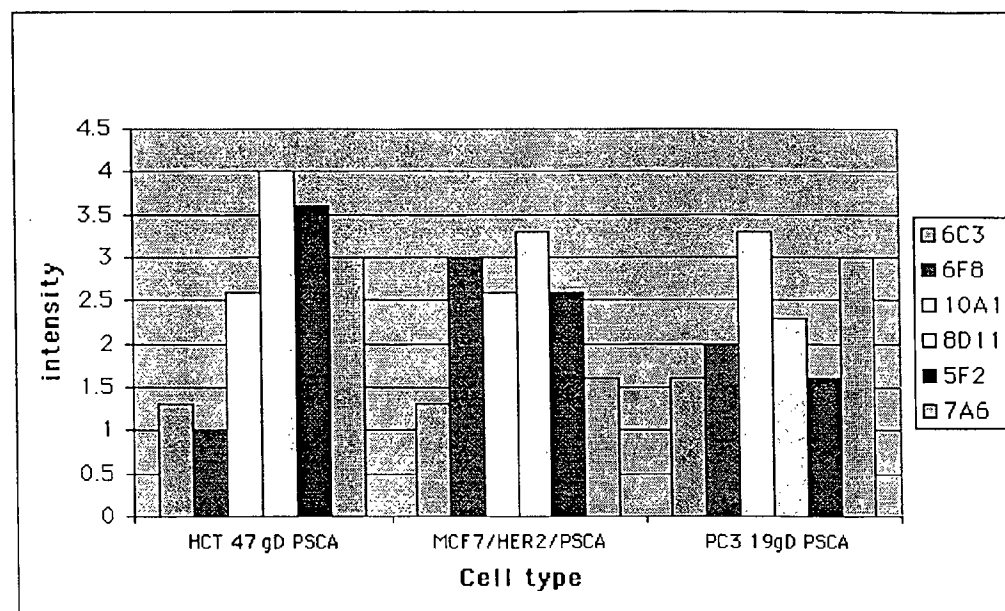


Fig. 6

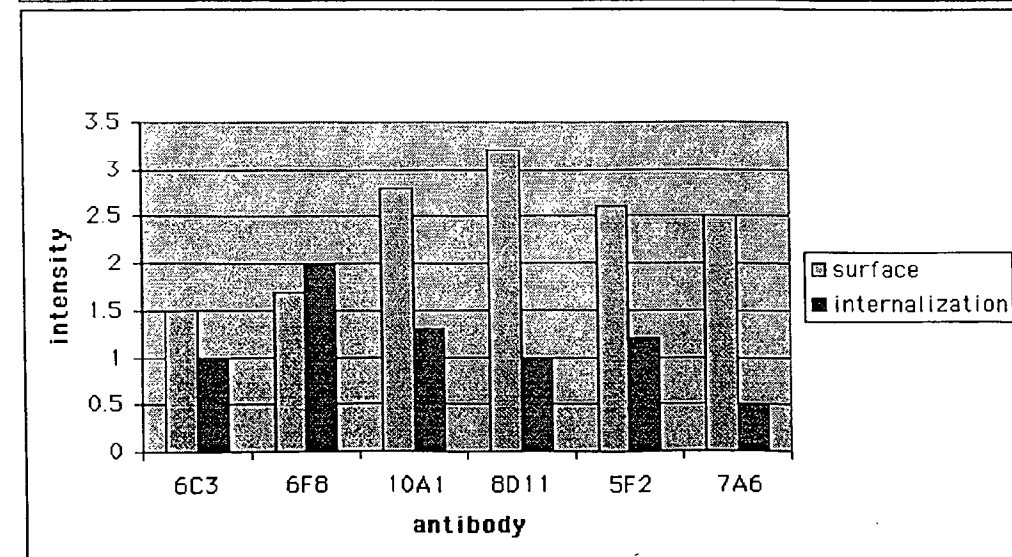


Fig. 7

FIG. 8A

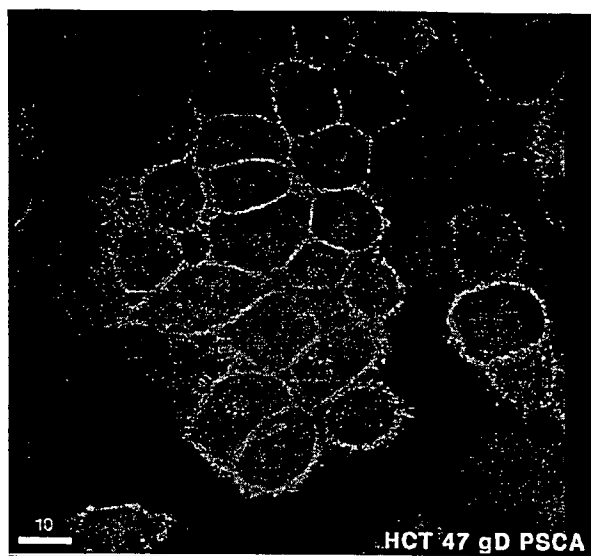


FIG. 8B

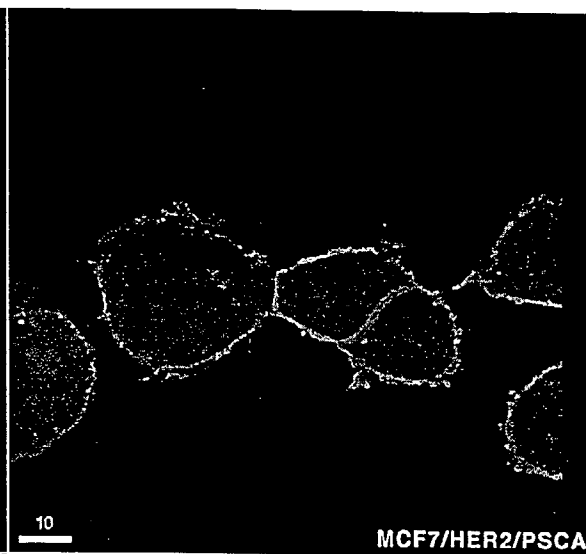
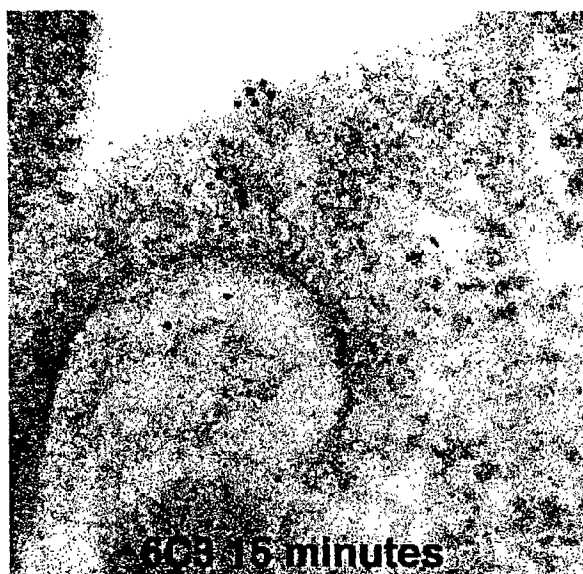


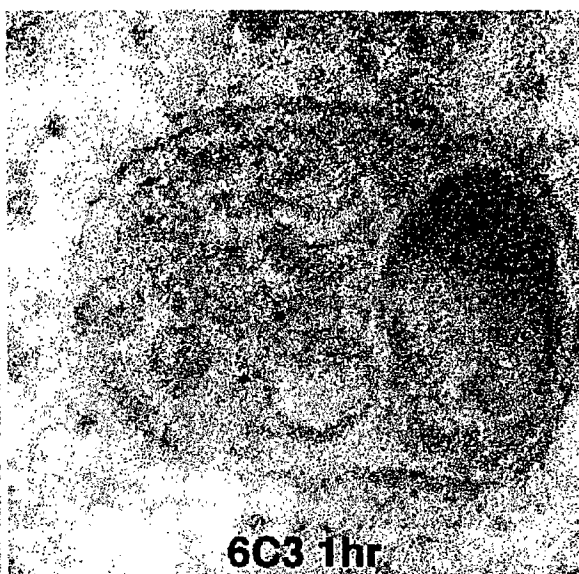
FIG. 8C

FIG. 9A

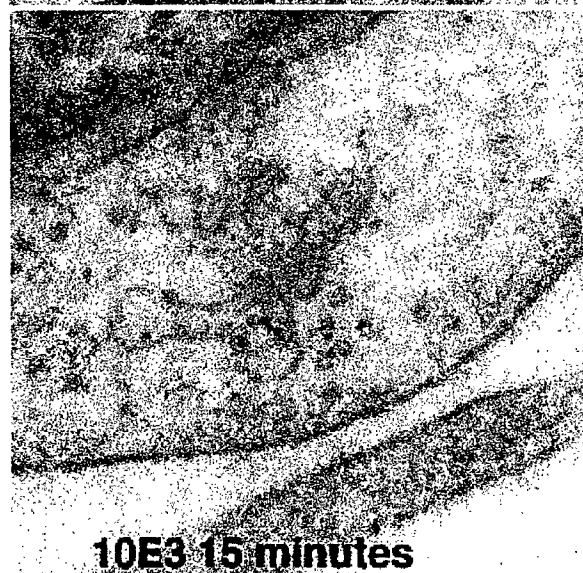


6C3 15 minutes

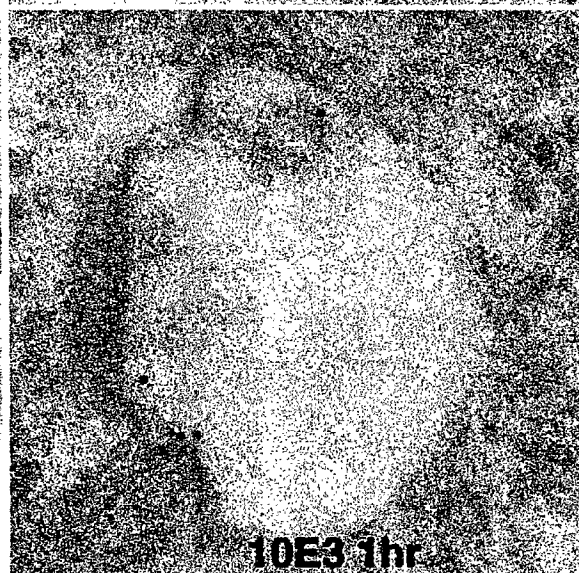
FIG. 9B



6C3 1hr



10E3 15 minutes



10E3 1hr

FIG. 9C

FIG. 9D



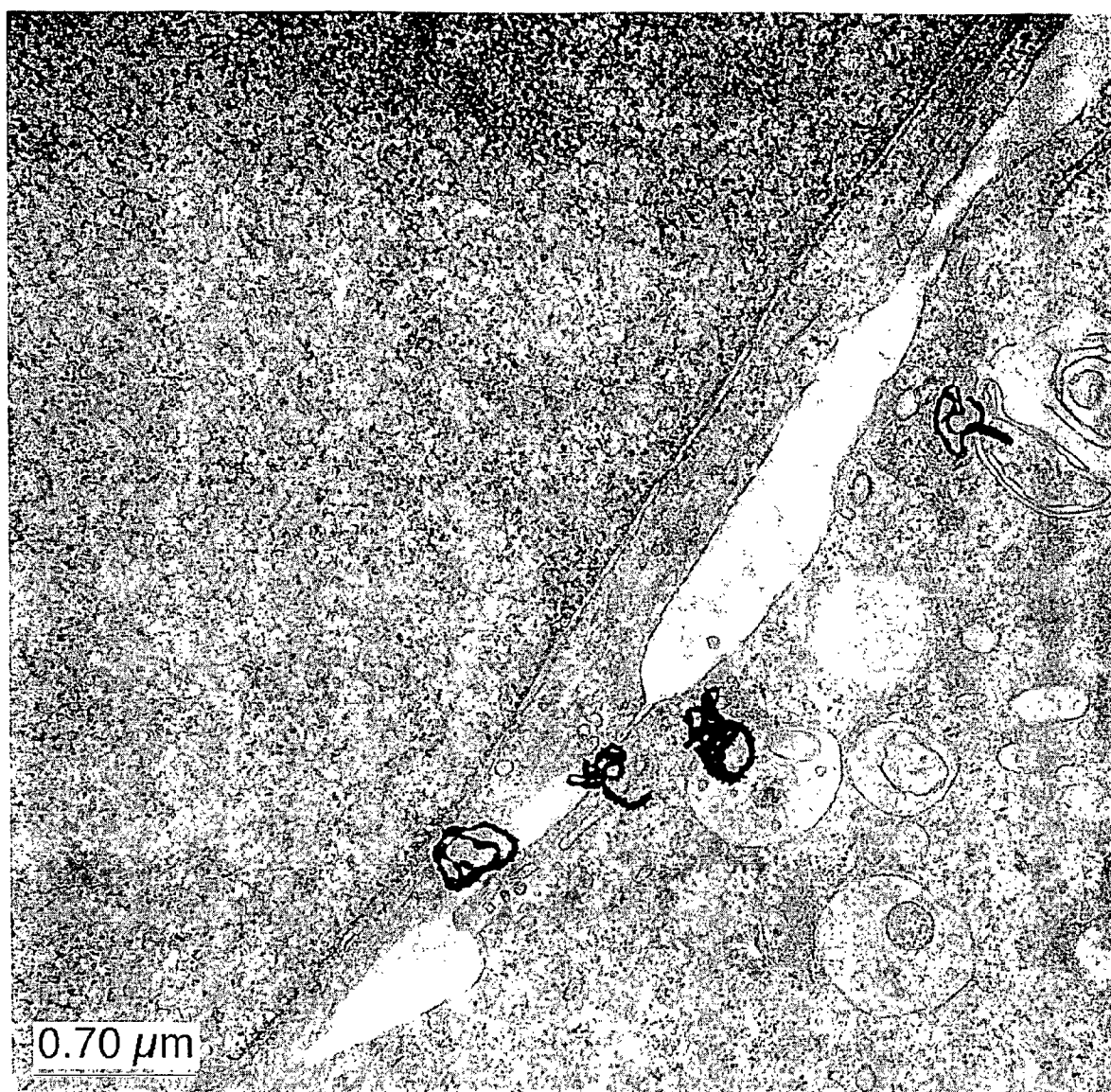


FIG. 10

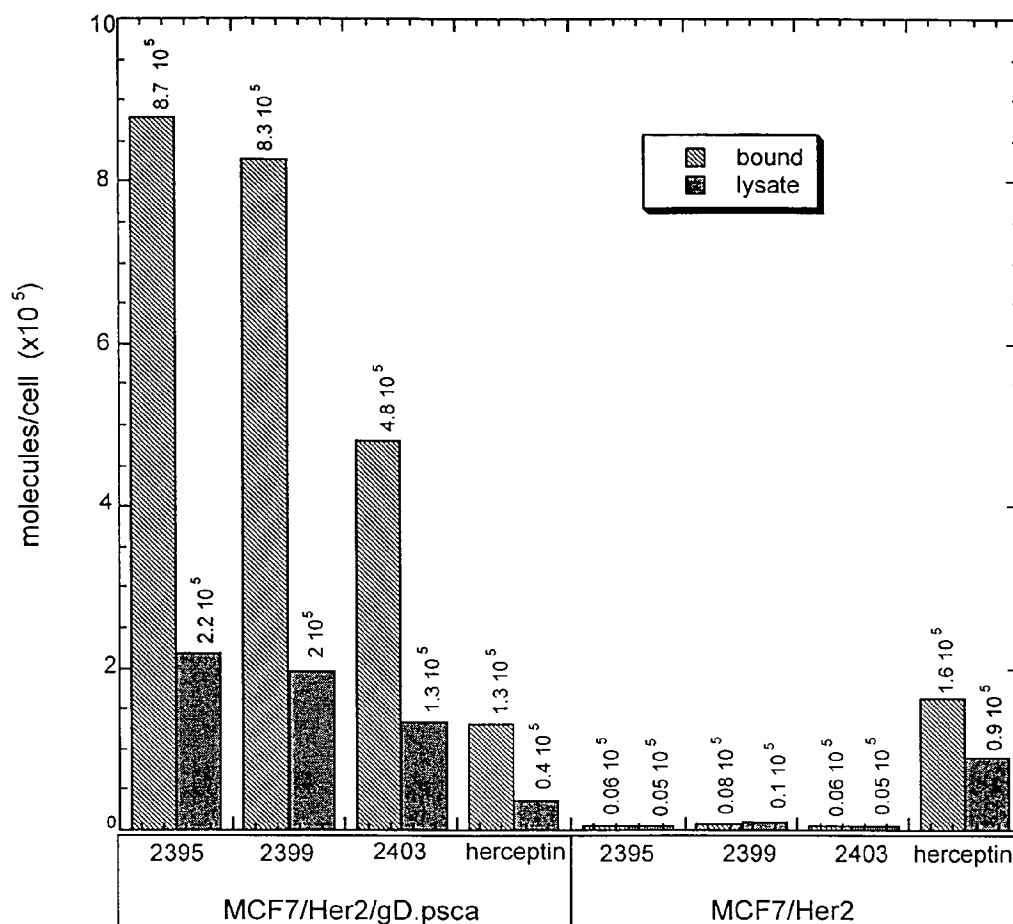


FIG. 11

Heavy chain sequences of anti-PSCA MAbs

2395 : N-----ELVKPGAPVKLSCKASGYTFNNYMNWVKQRPGRGLEWIGRIDPSXXXXTXXXQTFKDKA  
 2399 : N-----PGAELVKPGAPVKLSCKASGYTFNNYMNWVKQRPGRGLEWIGRIDPSDSETQYNQTFKDKA  
 2403 : N-QVQVQQPGAELVKPGAPVKLSCKASGYTFNNYWLWVKQRPGRGLEWIGRIDPSDSEIHYDQKFKDKA  
 2761 : N-EVQLQQSGPDLEKPGASVKISCKPSGNSFTGYIHWVKQSHGKSLEWIGRVDPNNGFTSYNQKFKGKA

CDR1CDR2CDR3

2395 : TLTVDKSSSTAYIQLSSLTSEDSAVYYCAITAAIAMDYWGQTSVTVSSAKTTGPS-C  
 2399 : TLTVDKSSSTAYIQLSSLTSEDSAVYYCAITAAIAMDYWGQTSVTVSSAKTTGPS-C  
 2403 : TLTVDKSSSTAYIQLSSLTSEDSAVYYCALTIYAMAYWGQTSVTVSSAKTTGPS-C  
 2761 : ILTVDKSSSTAYMELRSLTSEDSAVYYCVG-NFFDS--WGQGTTLTVSSAKTTGPS-C

Light chain sequences of anti-PSCA MAbs

2395 : N-----SVSISCRSSKSLLSNGNTYLYWFLQRPQGSPQLLIYRMSNLAGVPPDRFS  
 2403 : N-DIVMTQAAPSVPTPGESVSISCRSSKSLLSNGNTYLYWFLQRPQGSPQLLIYRMSNLAGVPPDRFS  
 2761 : N-DVVMTQTPLTSLSVTIGQPASISCKSSQSLDSDGKTYLWLLQRPQGSPKRLIYLVSTLDSGVPPDRFT

CDR1CDR2CDR3

2395 : GSGSGTVFTLIRSRVEAEDVGYYCMQHLESFFTFGSGGTKLEIKR-C  
 2403 : GSGSGTFTLIRSRVEAEDVGYYCLQHLEYPYTFGGGTKLEIKR-C  
 2761 : GSGSGTDFTLKISRVEAEDLGYYCWWQGTHFPRTFGGGGTKLEIKR-C

FIG. 12

**chimeric 2403 (5F2.4H4.1E3) Light Chain**

signal peptide MGWSCILFLVATATGVHS  
 DIVMTQAAPSVPTGESVSISCRSSKSLLSHNGNTYLWFLQRPGQSPQLLIYRMSNLASGVDPDRFSGSGGTAFTLRISRVEAEDVGV  
 VYVCLQHLEYPYTFGGGKLELK/RTVAAPSVFIFPPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS  
 TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

**chimeric 2403 (5F2.4H4.1E3) IgG Heavy Chain**

signal peptide MGWSCILFLVATATGVHS  
 QVQVQQPGAELVKPGAPVKLSCKASGYFTFTNYWLNWVKQRPGRGLEWIGRIDPSDSEIHVDQKFKDKATLTVDKSSSTAYIQLSSLT  
 SEDSAVYYCALTGİYAMAYWGQGSTVTVSSAKTTG/PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL  
 QSSGLYSLSSVTVPSSSLGTQTYICNVNHHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVV  
 DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV  
 LPPSRREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYT  
 QKSLSLSPGK

**chimeric 2761 (6B8.1D7.2B3) Fab - Light Chain**

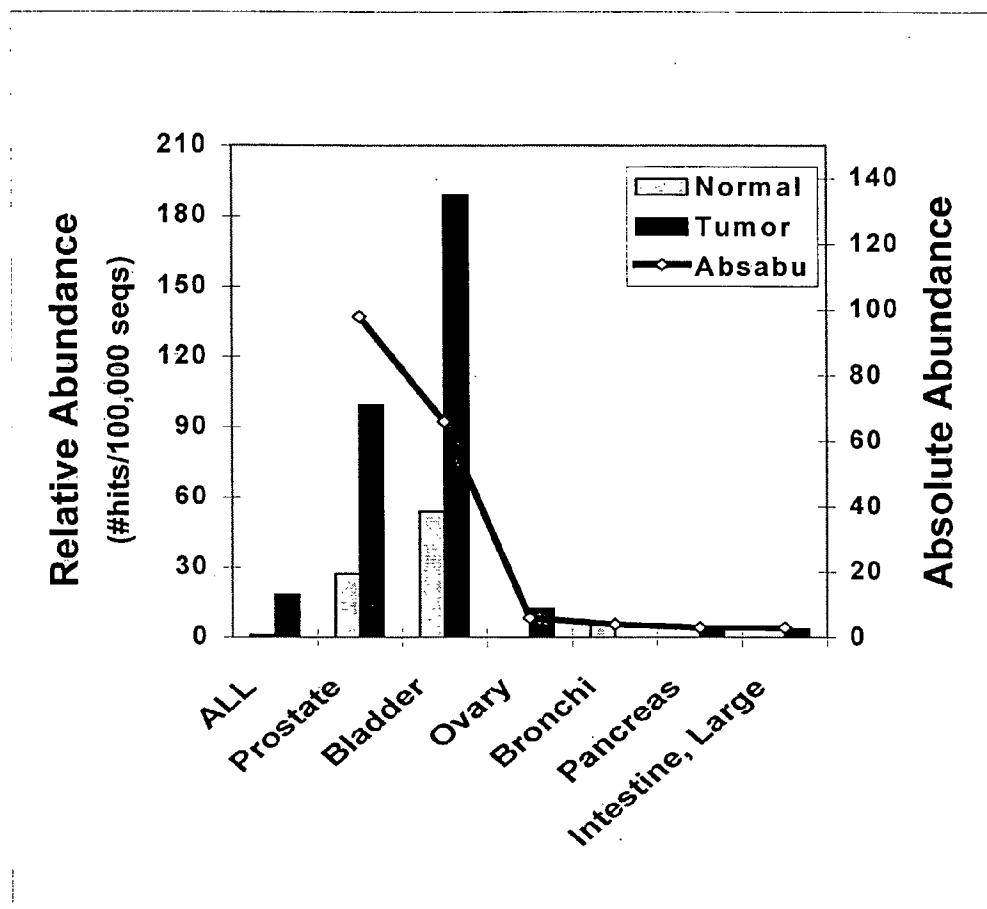
DVVMQTQPLTLSVTIGQPASISCKSSQSLDSDGKTYLNLWLLQRPQQPKRLIYLVSTLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGV  
 YYCWQGTHTFPRTFGGGKLEIKR [V<sub>L</sub>/C<sub>κ</sub> junction]  
 TVAAPSVFIFPPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTSLSKADYEKHKVYACET  
 HQGLSSPVTKSFNRGEC

**chimeric 2761 (6B8.1D7.2B3) Fab - Heavy chain**

EVQLQQSGPDLKPKGASVKISKCPSGNSFTGYIHWVKQSHGKSLEWIGRVDPNNGFTSYNQKFKGKAILTVDKSSSTAYMELRSLTSE  
 DSAVYYCVGNFFDSWGQGTITLVSSA [V<sub>H</sub>/C<sub>γ</sub>1 junction]  
 KTTGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHHKPSN  
 TKVDKKVEPKSCDKTHT

**FIG. 13**

FIG. 14



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```

1 ATGAAGGCTG TGCTGCTTGC CCTGTTGATG GCAGGCTTGG CCCTGCAGCC AGGCACTGCC
  TACTTCCGAC ACGACGAACG GGACAACTAC CGTCCGAACC GGGACGTCGG TCCGTGACGG
1 M K A V L L A L L M A G L A L Q P G T A

61 CTGCTGTGCT ACTCCTGCAA GGCCCAAGTG AGCAACGAGG ACTGCCTGAA TGTGGAGAAC
  GACGACACGA TGAGGACGTT CCGGGTCCAC TCGTTGCTCC TGACGGACTT ACACCTCTTG
21 L L C Y S C K A Q V S N E D C L N V E N

121 TGCACGCAGC CGGAGGAGCA GTGCTGGAAC GAGCGCATCC GCGCCGTGGG CCTCCTGACC
  ACGTGCCTCG GCCTCCTCGT CACGACCTGG CTCGCGTAGG CGCGGCACCC GGAGGACTGG
41 C T Q P E E Q C W T E R I R A V G L L T

181 GTCATCAGCA AAGGCTGCAG CTCAAACTGC GTGGATGACT CACAGGACTA CTACGTGGGC
  CAGTAGTCGT TTCCGACGTC GAGTTTGACG CACCTACTGA GTGTCCTGAT GATGCACCCG
61 V I S K G C S S N C V D D S Q D Y Y V G

241 AAGAAGAACA TCACCTGCTG TGACACCGAC TTGTGCAACG CCAGCGGGGC CCATGCACTG
  TTCTTCTTGT AGTGGACGAC ACTGTGGCTG AACACGTTGC GGTCCGCCCC GTTACGTCAC
81 K K N I T C C D T D L C N A S G A H A L

301 CAGCCGGCTG CTGCCATCCT GGCACCTGCTC CCTGCACTCA GTCTGCTGCT TTGGAGCCCC
  GTCGCGCCGAC GACGGTAGGA CCGTGACGAG GGACGTGAGT CAGACGACGA AACCTCGGGG
101 Q P A A A I L A L L P A L S L L L W S P

361 AGACAGCTGT AG
  TCTGTCGACA TC
121 R Q L O

```

FIG. 15

```

1 ATGAAGGCTG TGCTGCTTGC CCTGTTGATG GCAGGCTTGG CCCTGCAGCC AGGCACTGCC
  TACTTCCGAC ACGACGAACG GGACAACTAC CGTCCGAACC GGGACGTCGG TCCGTGACGG
1 M K A V L L A L L M A G L A L Q P G T A

61 CTGCTGTGCT ACTCCTGCAA GGCCCAAGTG AGCAACGAGG ACTGCCTGAA TGTGGAGAAC
  GACAACACGA TGAGGACGTT CCGGGTCCAC TCGTTGCTCC TGACGGACTT ACACCTCTTG
21 L L C Y S C K A Q V S N E D C L N V E N

121 TGCACGCAGC CGGAGGAGCA GTGCTGGAAC GAGCGCATCC GCGCCGTGGG CCTCCTGACC
  ACGTGCCTCG GCCTCCTCGT CACGACCTGG CTCGCGTAGG CGCGGCACCC GGAGGACTGG
41 C T Q P E E Q C W T E R I R A V G L L T

181 GTCATCAGCA AAGGCTGCAG CTCAAACTGC GTGGATGACT CACAGGACTA CTACGTGGGC
  CAGTAGTCGT TTCCGACGTC GAGTTTGACG CACCTACTGA GTGTCCTGAT GATGCACCCG
61 V I S K G C S S N C V D D S Q D Y Y V G

241 AAGAAGAACA TCACCTGCTG TGACACCGAC TTGTGCAACG CCAGCGGGGC CCATGCACTG
  TTCTTCTTGT AGTGGACGAC ACTGTGGCTG AACACGTTGC GGTCCGCCCC GTTACGCGGAC
81 K K N I T C C D T D L C N A S G A H A L

301 CAGCCAGCTG CTGCCATCCT GGCACCTGCTC CCTGCACTCA GCCTGCTGCT TTGGGGCCCC
  GTCGCGTCGAC GACGGTAGGA CCGTGACGAG GGACGTGAGT CAGACGACGA AACCTCGGGG
101 Q P A A A I L A L L P A L S L L L W G P

361 AGACAGCTGT AG
  TCTGTCGACA TC
121 R Q L O

```

FIG. 16

	10	20	30	40	50
human	MKAVLLALLMAGLALQPGTALLCYSCKAQVSNEDECLQVENCTQLGEQCWT				
	*****.*****				
cynomolgus	MKAVLLALLMAGLALQPGTALLCYSCKAQVSNEDECLNVENCTQPEEQCWT				
	10	20	30	40	50
	60	70	80	90	100
human	ARIRAVGLLTVISKGCSLNCVDDSQDYVVGKKNITCCDTDLCNASGAHAL				
	*****				
cynomolgus	ERIRAVGLLTVISKGCSLNCVDDSQDYVVGKKNITCCDTDLCNASGAHAL				
	60	70	80	90	100
	110	120			
human	QPAAAILALLPALGLLLWGPGQL				
	*****.****.* **				
cynomolgus	QPAAAILALLPALSLLLWSPRQL				
	110	120			

FIG. 17

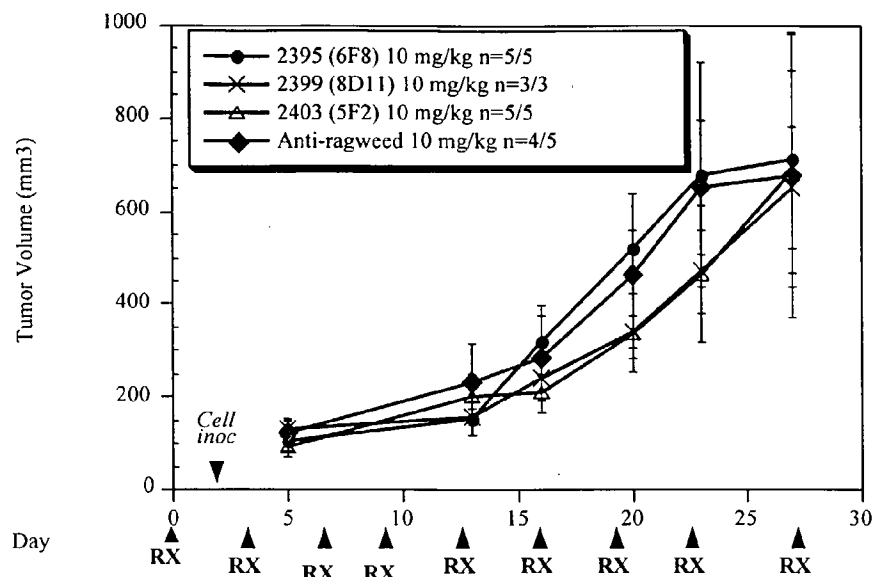


FIG. 18

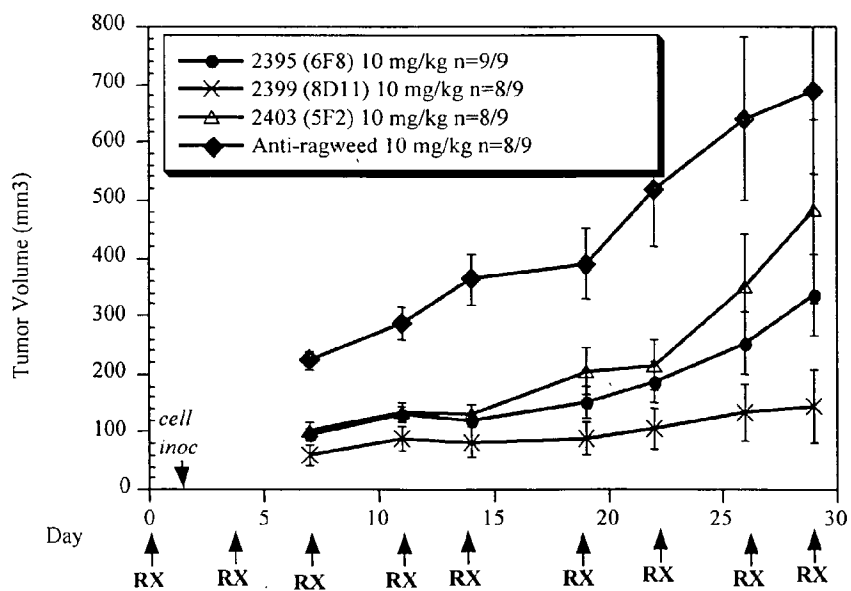


FIG. 19



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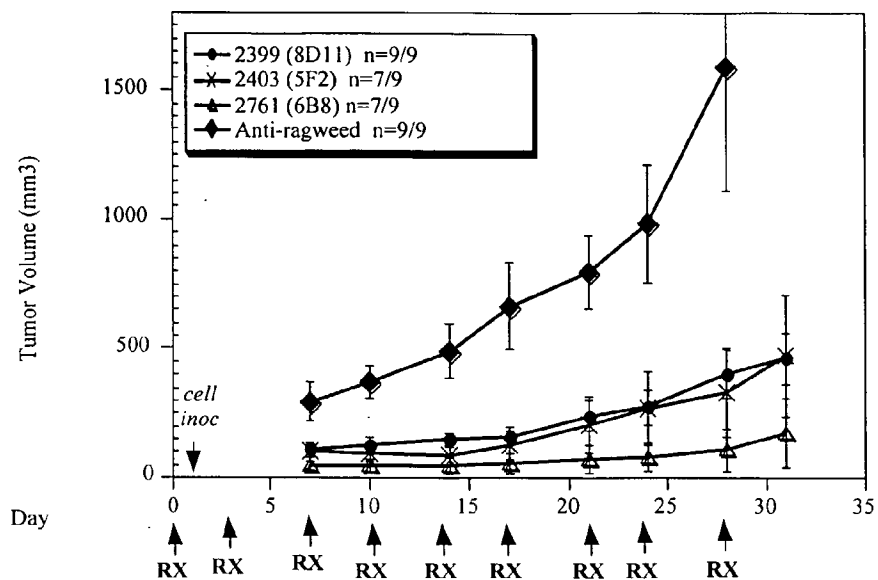


FIG. 20

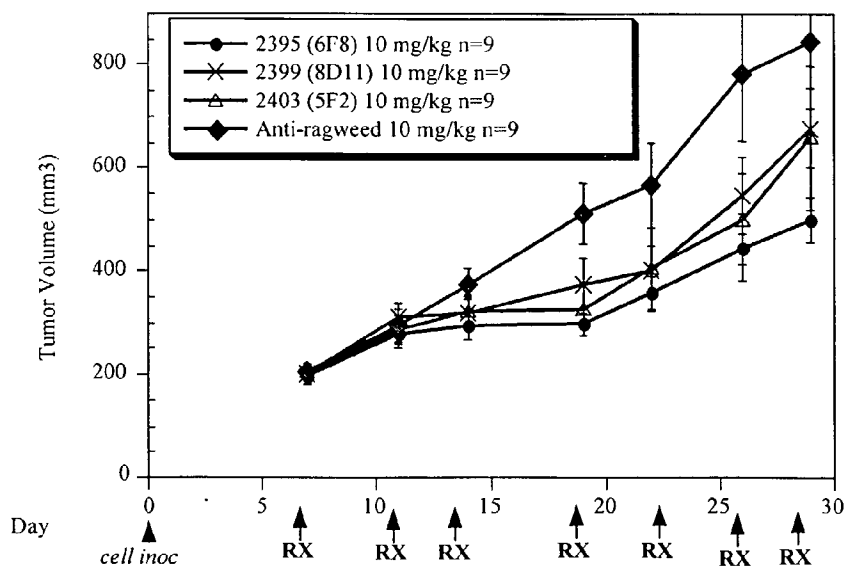


FIG. 21

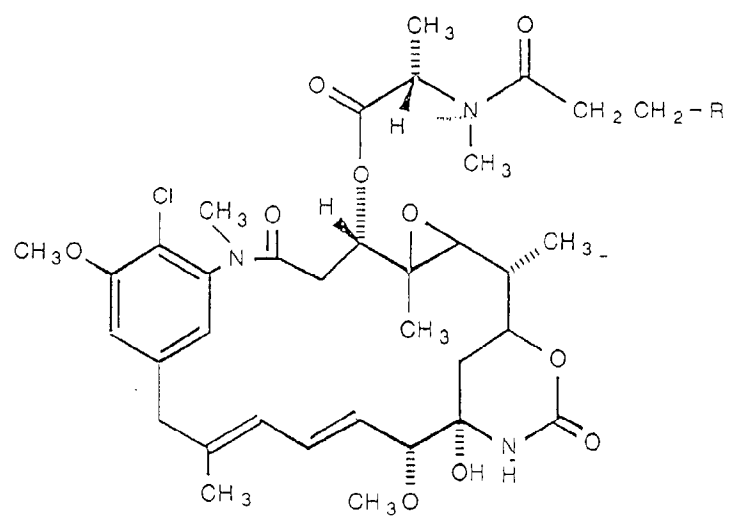


FIG 22

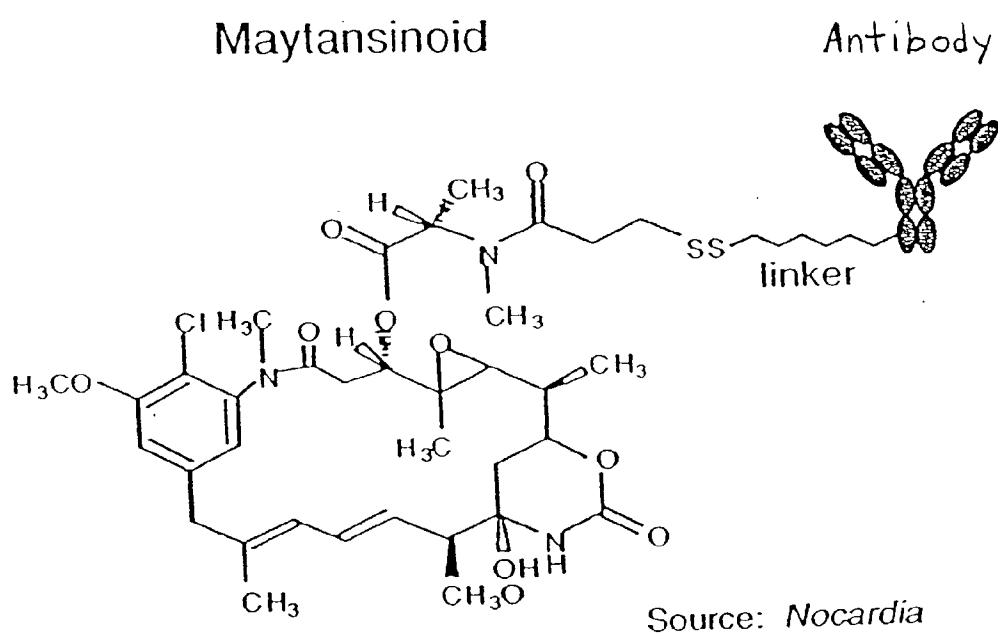


FIG. 23

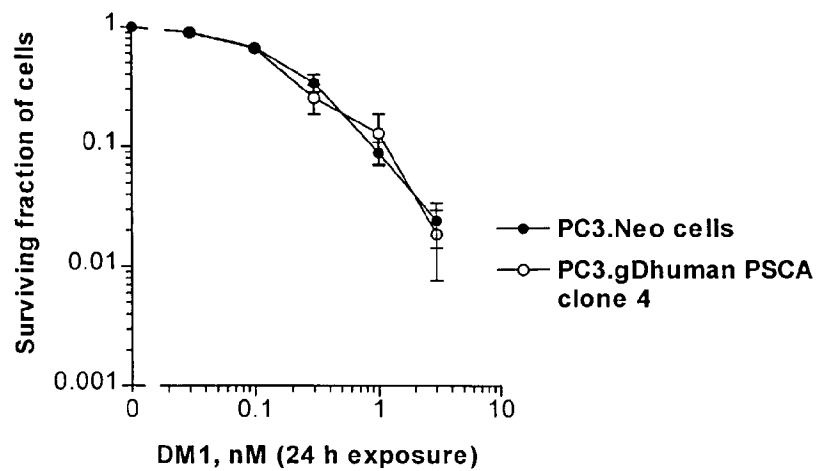


FIG. 24

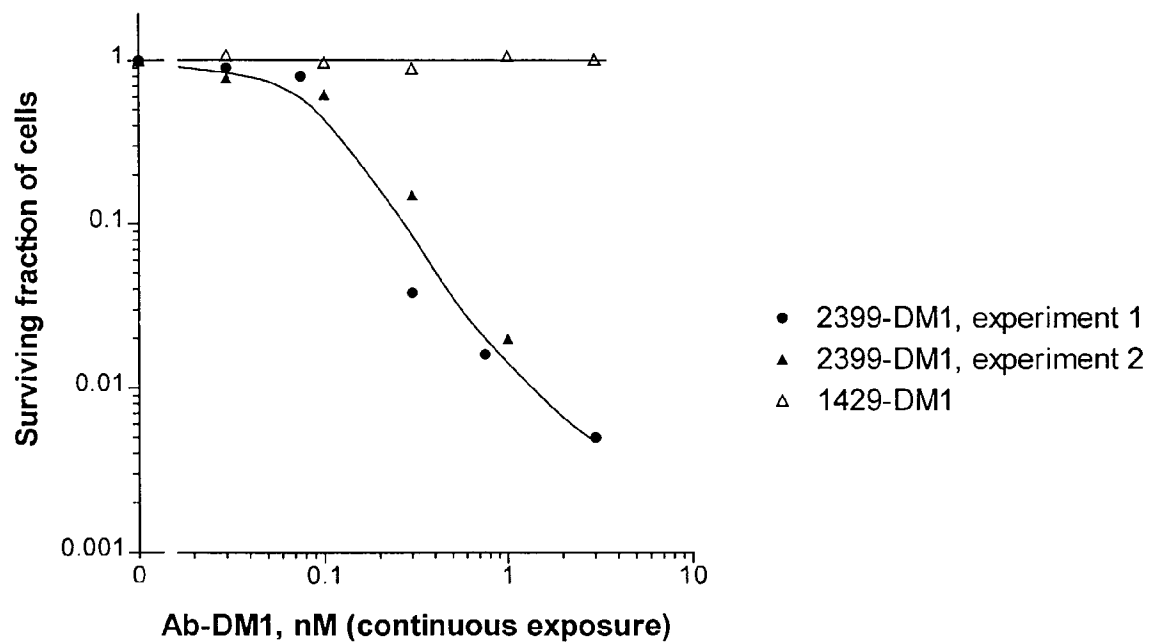


FIG. 25

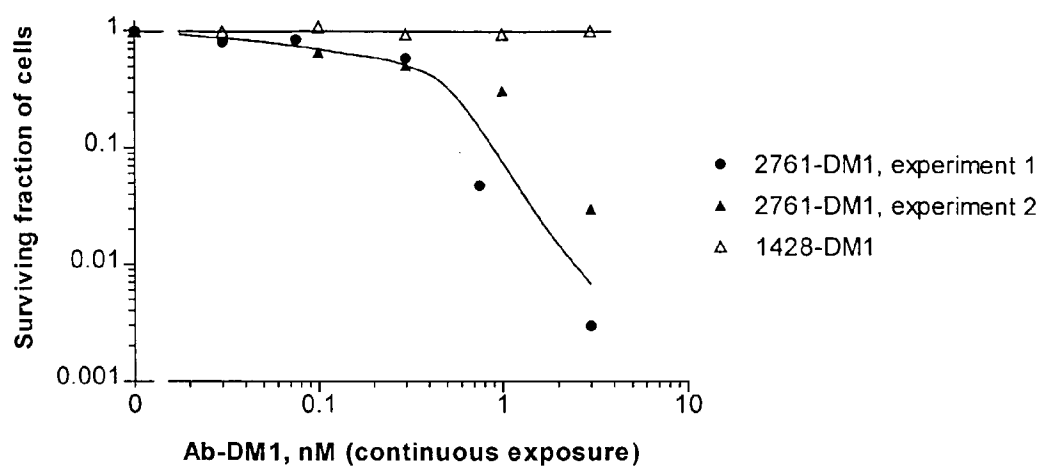


FIG. 26

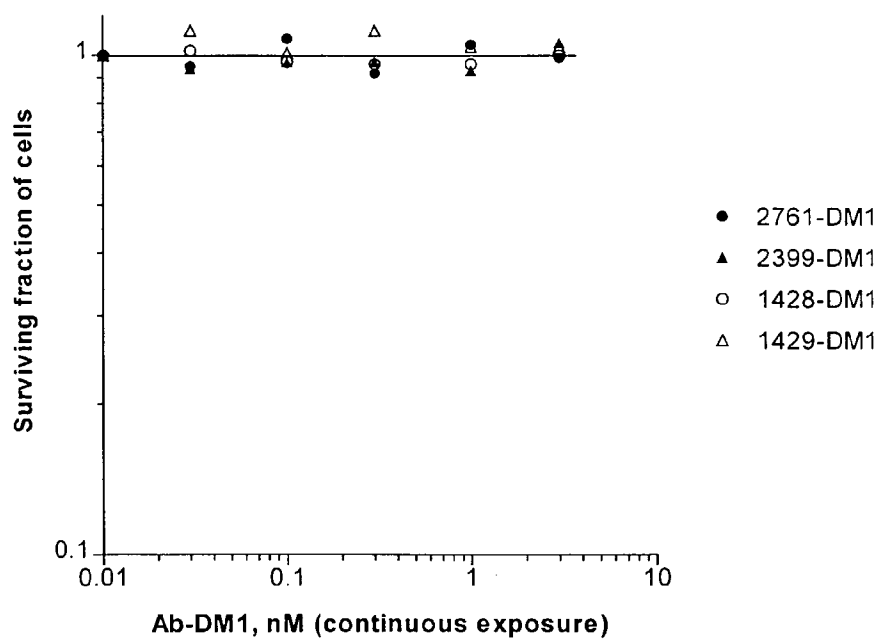
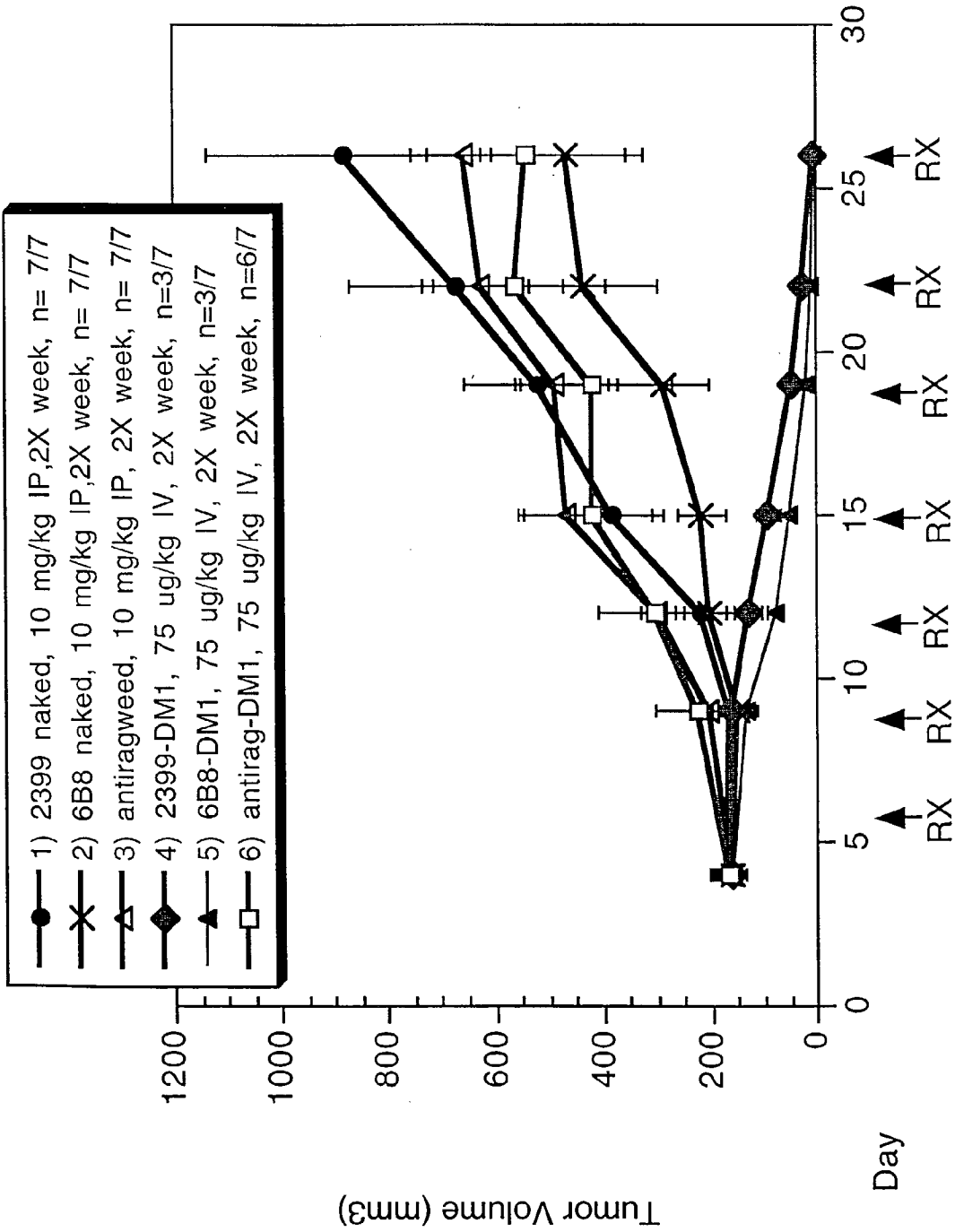


FIG. 27

FIG. 28



## Sequence Listing

<110> Genentech, Inc.  
 Devaux, Brigitte  
 Keller, Gilbert-Andre.  
 Koeppen, Hartmut  
 Lasky, Laurence A.

<120> Anti-Tumor Antibody Compositions and Methods of Use

<130> P1777R1PCT

<141> 2000-10-27

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<150> US 60/182,872  
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 35 40 45  
 Glu Gln Cys Trp Thr Ala Arg Ile Arg Ala Val Gly Leu Leu Thr  
 50 55 60  
 Val Ile Ser Lys Gly Cys Ser Leu Asn Cys Val Asp Asp Ser Gln  
 65 70 75  
 Asp Tyr Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp  
 80 85 90  
 Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala Ala Ala  
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 Glu Asn Cys Thr Gln Leu Gly Glu Gln Cys Trp Thr Ala  
           40                                  45                                  50

cgc atc cgc gca gtt ggc ctc ctg acc gtc atc agc aaa 192  
 Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile Ser Lys  
                                   55                                  60

ggc tgc agc ttg aac tgc gtg gat gac tca cag gac tac 231  
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           65                                  70                                  75

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Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr  
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Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr  
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 His Ser Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro  
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 Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro  
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 Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp  
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 Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val  
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 1 5 10 15  
 Ala Ser Val Lys Ile Ser Cys Lys Pro Ser Gly Asn Ser Phe Thr  
 20 25 30  
 Gly Tyr Tyr Ile His Trp Val Lys Gln Ser His Gly Lys Ser Leu  
 35 40 45  
 Glu Trp Ile Gly Arg Val Asp Pro Asn Asn Gly Phe Thr Ser Tyr  
 50 55 60  
 Asn Gln Lys Phe Lys Gly Lys Ala Ile Leu Thr Val Asp Lys Ser  
 65 70 75  
 Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp  
 80 85 90  
 Ser Ala Val Tyr Tyr Cys Val Gly Asn Phe Phe Asp Ser Trp Gly  
 95 100 105  
 Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Gly Pro  
 110 115 120  
 Ser

<210> 9  
 <211> 118  
 <212> PRT  
 <213> Mus musculus

<400> 9  
 Pro Gly Ala Glu Leu Val Lys Pro Gly Ala Pro Val Lys Leu Ser  
 1 5 10 15  
 Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Trp Met Asn Trp  
 20 25 30  
 Val Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile Gly Arg Ile  
 35 40 45  
 Asp Pro Ser Asp Ser Glu Thr Gln Tyr Asn Gln Thr Phe Lys Asp  
 50 55 60  
 Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Ile  
 65 70 75  
 Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 80 85 90  
 Ala Ile Thr Ala Ala Ile Ala Met Asp Tyr Trp Gly Gln Gly Thr  
 95 100 105  
 Ser Val Thr Val Ser Ser Ala Lys Thr Thr Gly Pro Ser  
 110 115

<210> 10  
 <211> 238

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; sequence is chimeric mouse/human

&lt;400&gt; 10

Met	Gly	Trp	Ser	Cys	Ile	Ile	Leu	Phe	Leu	Val	Ala	Thr	Ala	Thr
1				5					10					15

Gly	Val	His	Ser	Asp	Ile	Val	Met	Thr	Gln	Ala	Ala	Pro	Ser	Val
				20					25					30

Pro	Val	Thr	Pro	Gly	Glu	Ser	Val	Ser	Ile	Ser	Cys	Arg	Ser	Ser
				35					40					45

Lys	Ser	Leu	Leu	His	Ser	Asn	Gly	Asn	Thr	Tyr	Leu	Tyr	Trp	Phe
				50					55					60

Leu	Gln	Arg	Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Arg	Met
				65					70					75

Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly
				80					85					90

Ser	Gly	Thr	Ala	Phe	Thr	Leu	Arg	Ile	Ser	Arg	Val	Glu	Ala	Glu
				95					100					105

Asp	Val	Gly	Val	Tyr	Tyr	Cys	Leu	Gln	His	Leu	Glu	Tyr	Pro	Tyr
				110					115					120

Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Arg	Thr	Val	Ala
				125					130					135

Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys
				140					145					150

Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro
				155					160					165

Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser
				170					175					180

Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser
				185					190					195

Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr
				200					205					210

Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu
				215					220					225

Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys
				230					235			

&lt;210&gt; 11

&lt;211&gt; 466

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; sequence is chimeric mouse/human

&lt;400&gt; 11

Met	Gly	Trp	Ser	Cys	Ile	Ile	Leu	Phe	Leu	Val	Ala	Thr	Ala	Thr	
1				5					10					15	
Gly	Val	His	Ser	Gln	Val	Gln	Val	Gln	Gln	Pro	Gly	Ala	Glu	Leu	
				20					25					30	
Val	Lys	Pro	Gly	Ala	Pro	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Gly	
				35					40					45	
Tyr	Thr	Phe	Thr	Asn	Tyr	Trp	Leu	Asn	Trp	Val	Lys	Gln	Arg	Pro	
				50					55					60	
Gly	Arg	Gly	Leu	Glu	Trp	Ile	Gly	Arg	Ile	Asp	Pro	Ser	Asp	Ser	
				65					70					75	
Glu	Ile	His	Tyr	Asp	Gln	Lys	Phe	Lys	Asp	Lys	Ala	Thr	Leu	Thr	
				80					85					90	
Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Ile	Gln	Leu	Ser	Ser	Leu	
				95					100					105	
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Leu	Thr	Gly	Ile	
				110					115					120	
Tyr	Ala	Met	Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	
				125					130					135	
Ser	Ala	Lys	Thr	Thr	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	
				140					145					150	
Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	
				155					160					165	
Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	
				170					175					180	
Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	
				185					190					195	
Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	
				200					205					210	
Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	
				215					220					225	
Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	
				230					235					240	
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	
				245					250					255	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	
				260					265					270	
Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	
				275					280					285	
Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	
				290					295					300	
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	
				305					310					315	
Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	

	320		325		330
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys	335		340		345
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly	350		355		360
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu	365		370		375
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly	380		385		390
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln	395		400		405
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp	410		415		420
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg	425		430		435
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala	440		445		450
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly	455		460		465

Lys

&lt;210&gt; 12

&lt;211&gt; 218

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; sequence is chimeric mouse/human

&lt;400&gt; 12

Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val Thr Ile	1	5	10	15
Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu	20	25	30	
Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro	35	40	45	
Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Thr Leu Asp	50	55	60	
Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp	65	70	75	
Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val	80	85	90	
Tyr Tyr Cys Trp Gln Gly Thr His Phe Pro Arg Thr Phe Gly Gly	95	100	105	
Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val	110	115	120	

Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	
				125						130				135	
Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	
				140					145					150	
Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	
				155					160					165	
Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	
				170					175					180	
Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	
				185					190					195	
Val	Tyr	Ala	Cys	Glu	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	
				200					205					210	
Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys								
				215											

&lt;210&gt; 13

&lt;211&gt; 222

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; sequence is chimeric mouse/human

&lt;400&gt; 13

Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Asp	Leu	Glu	Lys	Pro	Gly	
1				5					10					15	
Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Pro	Ser	Gly	Asn	Ser	Phe	Thr	
				20					25					30	
Gly	Tyr	Tyr	Ile	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu	
				35					40					45	
Glu	Trp	Ile	Gly	Arg	Val	Asp	Pro	Asn	Asn	Gly	Phe	Thr	Ser	Tyr	
				50					55					60	
Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Ile	Leu	Thr	Val	Asp	Lys	Ser	
				65					70					75	
Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	
				80					85					90	
Ser	Ala	Val	Tyr	Tyr	Cys	Val	Gly	Asn	Phe	Phe	Asp	Ser	Trp	Gly	
				95					100					105	
Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Ala	Lys	Thr	Thr	Gly	Pro	
				110					115					120	
Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	
				125					130					135	
Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	
				140					145					150	
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	
				155					160					165	
Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	

	170		175		180
Ser Val Val Thr	Val Pro Ser Ser Ser	Leu Gly Thr Gln Thr Tyr			
	185	190			195
Ile Cys Asn Val	Asn His Lys Pro Ser	Asn Thr Lys Val Asp Lys			
	200	205			210
Lys Val Glu Pro	Lys Ser Cys Asp Lys	Thr His Thr			
	215	220			

&lt;210&gt; 14

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Sequence is a primer

&lt;400&gt; 14

aaggctgtgc tgcttgccct 20

&lt;210&gt; 15

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Sequence is a primer

&lt;400&gt; 15

gagtggcaca aaggcctggg 20

&lt;210&gt; 16

&lt;211&gt; 372

&lt;212&gt; DNA

&lt;213&gt; Macaca fascicularis

&lt;400&gt; 16

atg aag gct gtg ctg ctt gcc ctg ttg atg gca ggc	36
Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly	
1 5 10	

ttg gcc ctg cag cca ggc act gcc ctg ctg tgc tac tcc	75
Leu Ala Leu Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser	
15 20 25	

tgc aag gcc cag gtg agc aac gag gac tgc ctg aat gtg	114
Cys Lys Ala Gln Val Ser Asn Glu Asp Cys Leu Asn Val	
30 35	

gag aac tgc acg cag ccg gag gag cag tgc tgg acc gag	153
Glu Asn Cys Thr Gln Pro Glu Glu Gln Cys Trp Thr Glu	
40 45 50	

cgc atc cgc gcc gtg ggc ctc ctg acc gtc atc agc aaa	192
Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile Ser Lys	
55 60	

ggc tgc agc tca aac tgc gtg gat gac tca cag gac tac	231
Gly Cys Ser Ser Asn Cys Val Asp Asp Ser Gln Asp Tyr	
65 70 75	

tac gtg ggc aag aag aac atc acc tgc tgt gac acc gac	270
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Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp  
           80                                  85                                  90  
 ttg tgc aac gcc agc ggg gcc cat gca ctg cag ccg gct 309  
 Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala  
                           95                                  100  
 gct gcc atc ctg gca ctg ctc cct gca ctc agt ctg ctg 348  
 Ala Ala Ile Leu Ala Leu Leu Pro Ala Leu Ser Leu Leu  
           105                                  110                                  115  
 ctt tgg agc ccc aga cag ctg t ag 372  
 Leu Trp Ser Pro Arg Gln Leu  
                   120                                  123

<210> 17  
 <211> 123  
 <212> PRT  
 <213> Macaca fascicularis

<400> 17  
 Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly Leu Ala Leu  
   1                          5                                  10                                  15  
 Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser Cys Lys Ala Gln Val  
                           20                                  25                                  30  
 Ser Asn Glu Asp Cys Leu Asn Val Glu Asn Cys Thr Gln Pro Glu  
                           35                                  40                                  45  
 Glu Gln Cys Trp Thr Glu Arg Ile Arg Ala Val Gly Leu Leu Thr  
                           50                                  55                                  60  
 Val Ile Ser Lys Gly Cys Ser Ser Asn Cys Val Asp Asp Ser Gln  
                           65                                  70                                  75  
 Asp Tyr Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp  
                           80                                  85                                  90  
 Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala Ala Ala  
                           95                                  100                                  105  
 Ile Leu Ala Leu Leu Pro Ala Leu Ser Leu Leu Leu Trp Ser Pro  
                           110                                  115                                  120  
 Arg Gln Leu

<210> 18  
 <211> 372  
 <212> DNA  
 <213> Macaca fascicularis

<400> 18  
   atg aag gct gtg ctg ctt gcc ctg ttg atg gca ggc 36  
 Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly  
   1                          5                                  10  
 ttg gcc ctg cag cca ggc act gcc ctg ttg tgc tac tcc 75  
 Leu Ala Leu Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser  
           15                                  20                                  25  
 tgc aag gcc cag gtg agc aac gag gac tgc ctg aat gtg 114  
 Cys Lys Ala Gln Val Ser Asn Glu Asp Cys Leu Asn Val

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          30                      35
gag aac tgc acg cag ccg gag gag cag tgc tgg acc gag 153
Glu Asn Cys Thr Gln Pro Glu Glu Gln Cys Trp Thr Glu
    40                      45                      50

cgc atc cgc gcc gtg ggc ctc ctg acc gtc atc agc aaa 192
Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile Ser Lys
    55                      60

ggc tgc agc tca aac tgc gtg gat gac tca cag gac tac 231
Gly Cys Ser Ser Asn Cys Val Asp Asp Ser Gln Asp Tyr
    65                      70                      75

tac gtg ggc aag aag aac atc acc tgc tgt gac acc gac 270
Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp
    80                      85                      90

ttg tgc aac gcc agc ggg gcc cat gcc ctg cag cca gct 309
Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala
    95                      100

gct gcc atc ctg gca ctg ctc cct gca ctc agc ctg ctg 348
Ala Ala Ile Leu Ala Leu Leu Pro Ala Leu Ser Leu Leu
    105                      110                      115

ctt tgg ggc ccc aga cag ctg t ag 372
Leu Trp Gly Pro Arg Gln Leu
    120                      123

<210> 19
<211> 123
<212> PRT
<213> Macaca fascicularis

<400> 19
Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly Leu Ala Leu
  1                      5                      10                      15

Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser Cys Lys Ala Gln Val
    20                      25                      30

Ser Asn Glu Asp Cys Leu Asn Val Glu Asn Cys Thr Gln Pro Glu
    35                      40                      45

Glu Gln Cys Trp Thr Glu Arg Ile Arg Ala Val Gly Leu Leu Thr
    50                      55                      60

Val Ile Ser Lys Gly Cys Ser Ser Asn Cys Val Asp Asp Ser Gln
    65                      70                      75

Asp Tyr Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp
    80                      85                      90

Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala Ala Ala
    95                      100                      105

Ile Leu Ala Leu Leu Pro Ala Leu Ser Leu Leu Leu Trp Gly Pro
    110                      115                      120

Arg Gln Leu

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<210> 20

<211> 35  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Sequence is a primer

<400> 20  
actatgaagc ttgcagctc atcccttcac aatcg 35

<210> 21  
<211> 42  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Sequence is a primer

<400> 21  
gaattcggat ccaccatgaa gaccgtcttc tttctoctgc tg 42

<210> 22  
<211> 17  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Sequence is a PCR primer

<400> 22  
cctgctggcc acctact 17

<210> 23  
<211> 18  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Sequence is a primer

<400> 23  
ccttcacaat cgggctat 18

<210> 24  
<211> 20  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Sequence is a PCR primer

<400> 24  
acccacgcgt ccggctgctt 20

<210> 25  
<211> 21  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Sequence is a PCR primer

<400> 25  
cgggggacac cacggaccag a 21

Applicant's or agent's file reference number <b>P1777R1</b>	International application No.
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

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A. The indications made below relate to the microorganism referred to in the description on page <u>73</u> , line s <u>38-41</u>	
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Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, VA 20110-2209</b>	
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(54) Title: ANTI-PROSTATE STEM CELL ANTIGEN (PSCA) ANTIBODY COMPOSITIONS AND METHODS OF USE

(57) Abstract: The invention provides isolated anti-prostate stem cell antigen (PSCA) antibodies that internalize upon binding to PSCA on a mammalian in vivo. The invention also encompasses compositions comprising an anti-PSCA antibody and a carrier. These compositions can be provided in an article of manufacture or a kit. Another aspect of the invention is an isolated nucleic acid encoding an anti-PSCA antibody, as well as an expression vector comprising the isolated nucleic acid. Also provided are cells that produce the anti-PSCA antibodies. The invention encompasses a method of producing the anti-PSCA antibodies. Other aspects of the invention are a method of killing a PSCA-expressing cancer cell, comprising contacting the cancer cell with an anti-PSCA antibody and a method of alleviating or treating a PSCA-expressing cancer in a mammal, comprising administering a therapeutically effective amount of the anti-PSCA antibody to the mammal.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/29603

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/30 C12N5/10 C12N5/16 C12N15/13 C12N15/67  
A61K39/395 A61K47/48 A61K51/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, BIOSIS, CHEM ABS Data, EPO-Internal, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 40403 A (UNIV CALIFORNIA) 17 September 1998 (1998-09-17) cited in the application  page 10, line 1 -page 14, line 17 page 23, line 18 -page 25, line 14 page 35, line 5 -page 37, line 35 ---	15, 20, 21, 29-32, 46-55
A	REITER R E ET AL: "Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 FEB 17) 95 (4) 1735-40. , XP002089381 the whole document ----- -/--	1-56

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

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\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

22 May 2001

Date of mailing of the international search report

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Name and mailing address of the ISA

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/29603

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 01 05427 A (UNIV CALIFORNIA ;UROGENESYS (US)) 25 January 2001 (2001-01-25) page 24, line 5 -page 35, line 25 page 51, line 17 -page 63, line 7 page 115, line 1 -page 124, line 10 page 139, line 1 -page 141, line 5 ----	1-56
T	SAFFRAN, DOUGLAS C. ET AL: "Anti- PSCA mAbs inhibit tumor growth and metastasis formation an prolong the survival of mice bearing human prostate cancer xenografts." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (FEBRUARY 27, 2001) VOL. 98, NO. 5, PP. 2658-2663. PRINT. , XP000999115 the whole document ----	15,20, 21, 29-32, 46-55
P,X	AMARA, NORDINE (1) ET AL: "Monoclonal antibodies against prostate stem cell antigen inhibit prostate cancer tumour formation i SCID mice." EUROPEAN UROLOGY, (MARCH, 2000) VOL. 37, NO. SUPPL. 2, PP. 105, ABSTRACT 420. PRINT. MEETING INFO.: XVTH CONGRESS OF THE EUROPEAN ASSOCIATION OF UROLOGY BRUSSELS, BELGIUM APRIL 12-15, 2000 , XP000999929 the whole document ----	15,20, 21, 29-32, 46-55
P,X	WO 00 32752 A (UNIV CALIFORNIA) 8 June 2000 (2000-06-08) page 17, line 23 -page 27, line 2 page 42, line 1 -page 46, line 11 -----	1-56

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/29603

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

Continuation of Box I.1

Although claims 34-45, insofar as the cancer cells to be treated have not been isolated from the body, and claims 46-55, as a whole, are directed to a method of treatment of the human/animal body by therapy, the search has been carried out and based on the alleged effects of the antibody.

# INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. .nal Application No

PCT/US 00/29603

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9840403 A	17-09-1998	AU 6548198 A EP 0981541 A	29-09-1998 01-03-2000
WO 0105427 A	25-01-2001	AU 6364100 A	05-02-2001
WO 0032752 A	08-06-2000	AU 2166800 A	19-06-2000

CORRECTED VERSION

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WO 01/040309 A3

## ANTI-PROSTATE STEM CELL ANTIGEN (PSCA) ANTIBODY COMPOSITIONS AND METHODS OF USE

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FIELD OF THE INVENTION

The present invention relates to anti-PSCA antibody compositions and methods of killing PSCA-expressing cancers cells.

BACKGROUND OF THE INVENTION

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In humans, prostate cancer is one of the most commonly diagnosed malignancies in males and is the second leading cause of cancer related death in men. The American Cancer Society estimates that for the year 2000, 180,400 new cases of prostate cancer will be diagnosed with 31,900 deaths from the disease. In advanced stages, prostate cancer metastasizes to the bone. While advances in early diagnosis and treatment of locally confined tumors have been achieved, prostate cancer is incurable once it has metastasized. Patients with metastatic prostate cancer on hormonal therapy will eventually develop an androgen-refractory (androgen independent) state that will lead to disease progression and death. Currently, prostate-specific antigen (PSA) is the most widely used tumor marker for screening, diagnosis, and monitoring prostate cancer. However, widespread use of PSA as a tool for screening is controversial since PSA fails to discriminate accurately between benign and malignant prostate disease.

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Depending on the stage of the cancer, prostate and bladder cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, chemotherapy, androgen deprivation (e.g., hormonal therapy) in the case of prostate cancer. While surgical or radiation therapy significantly improves survival in patients with early stages of the disease, the therapeutic options are very limited for advanced cases, particularly for tumor recurrences following hormone ablation. The majority of patients who undergo hormone therapy progress to develop androgen-independent disease. Currently, there is no effective treatment for the 20-40% of prostate cancer patients who develop recurrent disease after surgery or radiation therapy, or for those in whom the cancer has metastasized at the time of diagnosis. Chemotherapy has its toxic side effects, especially in elderly patients. Development of new forms of therapy especially for disease refractory to androgen deprivation is an urgent need in the management of prostatic carcinoma.

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The identification of a novel cell surface antigen, prostate stem cell antigen (PSCA) has been described, see, e.g., U.S. Patent 5,856,136 (SCAH2), WO 99/14328 (protein PRO232), WO 98/40403 (PSCA), WO 98/51805 (PS116) and Reiter et al. *Proc. Nat. Acad. Sci.* 95:1735-1740 (1998). PSCA was initially cloned from a cDNA library of a LAPC-4 xenograft from a prostate cancer patient.

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PSCA is a GPI-linked molecule of 123 amino acids that is expressed on the surface of a number of cell types including prostate and bladder tumor cells. The gene is located on the *myc* locus on 8Q24.2 which is a region amplified in 80% of prostate cancers. PSCA shows 30% homology with SCA-2. The protein has a hydrophobic signal sequence at the first 20 amino acids of the N-terminus and a GPI-anchoring sequence at amino acid 100-123 of the C-terminus (Fig. 2 on page 1737 of Reiter et al. (1998)). There are four predicted glycosylation sites. The cell surface protein is shed with a  $t_{1/2}$  of about 10 hours in culture. It has been reported that PSCA is widely over-expressed across all stages of prostate cancer, including high grade

prostatic intraepithelial neoplasia (PIN), and both androgen-dependent and -independent prostate tumors. Antibodies that are able to target PSCA-expressing tumor cells *in vivo* and that can internalize upon binding to the cells, have not been reported.

Antibody-based therapy has proved very effective in the treatment of various cancers. For example, HERCEPTIN® and RITUXAN® (both from Genentech, S. San Francisco), have been used successfully to treat breast cancer and non-Hodgkin's lymphoma, respectively. HERCEPTIN® is a recombinant DNA-derived humanized monoclonal antibody that selectively binds to the extracellular domain of the human epidermal growth factor receptor 2 (HER2) proto-oncogene. HER2 protein overexpression is observed in 25-30% of primary breast cancers. RITUXAN® is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. Both these antibodies are produced in CHO cells.

The present invention provides alternative methods of treating cancer that overcome the limitations of conventional therapeutic methods as well as offer additional advantages that will be apparent from the detailed description below.

#### SUMMARY OF THE INVENTION

The invention provides isolated anti-PSCA antibodies that internalize upon binding to PSCA on a mammalian cell *in vivo*. These antibodies can also target a PSCA-expressing tumor cell *in vivo*. In a specific embodiment, the anti-PSCA antibodies internalize upon binding to PSCA on cancer cells, including prostate cancer, urinary tract cancers (e.g., bladder cancer) and lung cancer. Provided are internalizing anti-PSCA antibodies that are monoclonal antibodies. In specific embodiments, the antibodies are 10E3, 6F8, 8D11, 5F2, 6C3, 6B8 and 10C5, produced by the hybridomas deposited under American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, and PTA-2264. The V region sequences of the 6F8, 8D11, 5F2, 6B8 antibodies are shown in Figures 12 (SEQ ID NOs. 3-9). The chimeric murine-human antibody polypeptides having the amino acid sequence of SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, or SEQ ID NO.13, are provided. The invention provides anti-PSCA antibodies and antibody fusion polypeptides that comprise the amino acid sequences of any one of SEQ ID NO. 1-13.

Also provided are antibodies that compete for binding to the same epitope as the epitope bound by any of the aforementioned monoclonal antibodies. In another embodiment, an isolated anti-PSCA monoclonal antibody that inhibits the growth of PSCA-expressing cancer cells *in vivo*, or is cytotoxic *in vivo*, to such cells and tumors containing such cells, is provided.

The invention also provides anti-PSCA antibodies that are conjugated to a cytotoxic agent or to a growth inhibitory agent. The antibodies are internalizing and/or growth inhibitory antibodies. The cytotoxic agent can be a toxin, antibiotic, radioactive isotope or nucleolytic enzyme. In a preferred embodiment, the toxin is a maytansinoid, more preferably the maytansinoid having the structure shown in FIG. 22.

The anti-PSCA antibodies of the preceding embodiments include intact (full length) antibodies as well as antibody fragments. The anti-PSCA antibodies of the invention include human antibodies and chimeric antibodies as well as antibody fusion polypeptides comprising at least the antibody V region sequences fused to a heterologous polypeptide. In a preferred embodiment, the anti-PSCA antibody of any of the preceding embodiments is a chimeric or human antibody. In a preferred embodiment, the chimeric

antibody is a humanized antibody. The humanized anti-PSCA antibodies include humanized forms of any of the antibodies produced by the hybridomas deposited under American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, and PTA-2265 and the chimeric antibodies include those having amino acid sequences of SEQ ID NO.10-13 as shown in FIG. 13. The antibodies of the invention include those produced in mammalian or bacterial cells.

The invention also encompasses a composition comprising any one of the anti-PSCA antibodies of the above embodiments, and a carrier. In one embodiment, the antibody in the composition is a human antibody or a humanized form of the monoclonal antibody produced by any one of the hybridomas deposited under ATCC accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, and PTA-2264. In one specific embodiment, the antibody in the composition is conjugated to a maytansinoid. In a preferred embodiment, the carrier is a pharmaceutically-acceptable carrier. These compositions can be provided in an article of manufacture or a kit.

Another aspect of the invention is an isolated nucleic acid encoding any one of the anti-PSCA antibodies of the above embodiments, as well as an expression vector comprising the isolated nucleic acid operably linked to an expression regulatory sequence.

Also provided by the invention are cells that produce the above-described anti-PSCA antibodies. In one embodiment, the antibody producing cells are hybridoma cells including the specific hybridoma cells having ATCC accession numbers PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, and PTA-2264. In another embodiment, the cell is a bacterial cell. A host cell comprising the above-described vector is specifically provided.

The invention further encompasses a method of producing the anti-PSCA antibodies of the above embodiments, comprising culturing the cells of the above embodiments and recovering the antibody from the cell culture.

Yet a separate aspect of the invention is a method of killing a PSCA-expressing cancer cell, comprising contacting the cancer cell with an anti-PSCA antibody of any of the above embodiments, thereby killing the cancer cell. Another aspect is a method of alleviating or treating a PSCA-expressing cancer in a mammal, comprising administering a therapeutically effective amount of the anti-PSCA antibodies of the invention to the mammal. In preferred embodiments of the preceding two methods, the cancer is a prostate, bladder or lung cancer, more preferably prostate cancer and especially an androgen independent prostate cancer cell or a metastatic prostate cancer. In a preferred embodiment of these methods, the anti-PSCA antibody is a human or a humanized antibody. In another preferred embodiment, the antibody is conjugated to a cytotoxic agent such as a toxin or a radioactive isotope. Preferably, the toxin is calicheamicin or a maytansinoid such as "DM1" having the structure shown in Fig. 22. The method of alleviating the PSCA-expressing cancer anticipates administration of the anti-PSCA antibody in conjunction with chemotherapy wherein the mammal is also receiving at least one chemotherapeutic agent. In a specific embodiment, the chemotherapeutic agent is a taxane such as paclitaxel (TAXOL®) or docetaxel, or derivatives and analogs thereof.

In a further aspect, the invention provides an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an anti-PSCA antibody of the above

embodiments, and further comprising a package insert indicating that the composition can be used to alleviate or treat a PSCA-expressing cancer and in particular, prostate cancer.

#### BRIEF DESCRIPTION OF THE FIGURES

5 Figure 1 shows the binding to and staining of gD-PSCA transfected CHO cells by Mab by 5F2, as assayed by FACS (see Example 1).

Figure 2 shows the binding to and staining of gD-PSCA transfected CHO cells by Mab by 10C5, as assayed by FACS (see Example 1).

10 Figure 3 shows the binding activity of the 6B8.1D7.2B3 anti-PSCA Mab to various soluble PSCA antigens (see Example 1).

Figure 4 shows the binding curve for antibody ascites no. 2403 (5F2) (see Example 1).

Figure 5 is a graph showing internalization of the different anti-PSCA monoclonal antibodies (6C3; 6F8; 10A1; 8D11; 5F2; 7A6) in the three cell types, as measured within 1 hour, by the intensity of the Cy3 fluorescence (see Example 3).

15 Figure 6 is a graph showing surface labeling of PSCA by the different anti-PSCA monoclonal antibodies (6C3; 6F8; 10A1; 8D11; 5F2; 7A6) on the three cell types, as measured by the intensity of the Cy3 fluorescence (see Example 3).

Figure 7 is a graph comparing the amount of surface versus internalized anti-PSCA monoclonal antibody for each of the antibodies as measured within 1 hour, by the intensity of the Cy3 fluorescence. The amount of surface and internalized antibody is an average of the results from the three different cell lines used in Figures 5 and 6.

Figures 8A, 8B and 8C are micrographs showing the distribution of the fluorescent labeled 8D11 anti-PSCA antibody in PSCA-expressing cell lines HCT 47 gD, MCF7/HER2/PSCA, and PC3 19 gD PSCA respectively, as observed by confocal microscopy (See Example 3).

25 Figures 9A-D show electron micrographs of CHO cells expressing PSCA treated with anti-PSCA antibodies labeled with gold adducts. Figures 9A and 9B show cells incubated with antibody 6C3 for 15 minutes and 1 hour, respectively. Figures 9C and 9D show cells incubated with antibody 10E3 for 15 minutes and 1 hour, respectively.

30 Figure 10 is a micrograph showing internalization of <sup>125</sup>I-8D11 antibody in HCT116 gDPSCA cells 15 minutes after contact with the antibody (see Example 3 below).

Figure 11 shows the levels of internalization of three anti-PSCA antibodies [ascites # 2395 (6F8), #2399 (8D11), #2403(5F2)] at 37°C after 5 hours, in Her2 -expressing MCF7 cells transfected with either gD-PSCA or vector alone (see Example 3 below). Herceptin, is an anti-Her2 antibody.

35 Figure 12 shows the amino acid sequences of the heavy and light chain variable region domains (V<sub>H</sub> and V<sub>L</sub>) of the antibodies from hybridoma clones 6F8.2F4, Asc# 2395; 8D11.2E9, Asc# 2399; 5F2.4H4.1E3, Asc# 2403; and 6B8.1D7.2B3, Asc#2761.

Figure 13 shows the amino acid sequences of the full length chimeric 2403 IgG including the signal peptide sequences, and the sequences of chimeric 6B8 Fab.

40 Figure 14 shows a comparison of the relative expression levels of PSCA in various tumor versus normal tissues (see Example 4).

Figure 15 shows the DNA and protein sequences of the Cynomolgus monkey PSCA type 1 (SEQ ID NO: 16 and NO:17, respectively), see Example 6. The "o" at the end of the amino acid sequence stands for the Ochre stop codon.

Figure 16 shows the DNA and protein sequences of the Cynomolgus monkey PSCA type II (SEQ ID NO: 18 and NO: 19, respectively), see Example 6. The "o" at the end of the amino acid sequence stands for the Ochre stop codon.

Figure 17 compares the amino acid sequences for human PSCA (SEQ ID NO.1) and Cynomolgus monkey type I PSCA (SEQ ID NO. 17).

Figure 18 shows the growth of control PC-3 tumor cells (PSCA-negative) in mice treated with 3 of the anti-PSCA antibodies (6B8, 8D11, and 5F2) as compared to the anti-ragweed negative control antibody (see Example 5).

Figure 19 shows the growth of PC-3.gDPSCA tumors in mice treated with 3 of the anti-PSCA antibodies (6F8, 8D11, and 5F2) as compared to the negative control, anti-ragweed antibody. The antibodies were administered by intraperitoneal injection starting one day prior to inoculation with PSCA-transfected PC-3 cells, followed by biweekly injections following cell inoculation. "RX" under the arrows indicates the time point (day) of injection of the antibodies into the mice (see Example 5).

Figure 20 shows the growth of PC-3.gDPSCA tumors treated with antibodies 8D11, 5F2, 6B8 and control ragweed antibodies, as described in Figure 19 (see Example 5).

Figure 21 shows the growth of PC-3.gDPSCA tumors in mice wherein treatment with anti-PSCA antibodies 6F8, 8D11, or 5F2 was initiated after the tumors had been allowed to grow for 1 week in the mice and had reached a mean tumor volume of approximately 200 mm<sup>3</sup> per group of mice. Antibody administration was biweekly. "RX" under the arrows indicates the time point (day) of intraperitoneal injection of the antibodies into the mice (see Example 5).

Figure 22 shows the structure of the maytansinoid designated "DM1" (see Example 7).

Figure 23 illustrates the structure of anti-PSCA antibody-DM1 conjugate (see Example 7).

Figure 24 shows the sensitivity of assay cell lines, PC3.Neo cells and PC3.gD human PSCA clone 4 cells, to non-conjugated DM1 (see Example 8).

Figure 25 shows the cytotoxicity of antibody conjugate 2399-DM1 to PC3.gDhuman PSCA clone 4 cells compared to isotype control 1429-DM1 (see Example 8).

Figure 26 shows the cytotoxicity of 2761-DM1 to PC3.gDhuman PSCA clone 4 cells compared to isotype control 1428-DM1 (see Example 8).

Figure 27 shows the lack of toxicity of antibody-DM1 conjugates in antigen-negative PC3.Neo cells (see Example 8).

Figure 28 shows the growth of PSCA-expressing, PC3 human prostate tumor cells in a mouse model in which the mice were treated with either naked (non-toxin conjugated) antibody or DM1-conjugated antibody. The anti-PSCA antibodies were 2399 or 6B8 and the anti-ragweed antibody 1428 serves as the control antibody (see Example 9).



DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**I. Definitions**

Human "PSCA" or "prostate stem cell antigen" as used herein, refers to a single subunit  
 5 glycoprotein of 123 amino acids that is expressed on the cell surface as a glycosylphosphatidylinositol (GPI)-  
 anchored protein, whose nucleotide and amino acid sequence sequences are as disclosed in e.g., U.S. Patent  
 5,856,136 (SCAH2; SEQ ID NO:4); WO 99/14328 (PRO232, nucleotide sequence in SEQ ID NO:1, Fig. 1;  
 amino acid sequence in Fig. 2; SEQ ID NO:2); Fig. 2 (amino acid sequence) on page 1737 of Reiter et al.  
*Proc. Nat. Acad. Sci.* 95:1735-1740 (1998); WO 98/51805 (SEQ ID NOs: 12 and 25); and WO 98/40403  
 10 (Figures 1A and 1B). The amino acid sequence of murine PSCA is provided in Fig. 2 on page 1737 of Reiter  
 et al. *Proc. Nat. Acad. Sci.* 95:1735-1740 (1998). The protein has a signal sequence at the amino-terminus  
 and the GPI-anchoring sequence is present at the carboxy terminus. In the human PSCA sequence, amino  
 acids 1-20 represent the N-terminal signal sequence which gets cleaved in the mature protein, and amino acid  
 positions 100-123 represent C-terminal GPI-anchoring sequences (Reiter et al., 1998, see Fig.2). Thus, the  
 15 amino acids 21 to about 100 are presumably on the cell surface. PSCA as used herein include allelic variants  
 and conservative substitution mutants of the protein which have PSCA biological activity. These allelic  
 variants and conservative substitution mutants may be GPI-linked or secreted forms of the protein.

The term "antibody" (Ab) as used herein includes monoclonal antibodies, polyclonal antibodies,  
 multispecific antibodies (e.g. bispecific antibodies), and antibody fragments, so long as they exhibit the  
 20 desired biological activity. The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

An "isolated antibody" is one which has been identified and separated and/or recovered from a  
 component of its natural environment. Contaminant components of its natural environment are materials  
 which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes,  
 hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will  
 25 be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most  
 preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or  
 internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE  
 under reducing or non-reducing conditions using Coomassie blue or, preferably, silver stain. Isolated  
 antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's  
 30 natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least  
 one purification step.

The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light  
 (L) chains and two identical heavy (H) chains (an IgM antibody consists of 5 of the basic heterotetramer unit  
 along with an additional polypeptide called J chain, and therefore contain 10 antigen binding sites, while  
 35 secreted IgA antibodies can polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain  
 units along with J chain). In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L  
 chain is linked to a H chain by one covalent disulfide bond, while the two H chains are linked to each other  
 by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly  
 spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain ( $V_H$ ) followed by  
 40 three constant domains ( $C_H$ ) for each of the  $\alpha$  and  $\gamma$  chains and four  $C_H$  domains for  $\mu$  and  $\epsilon$  isotypes. Each

L chain has at the N-terminus, a variable domain ( $V_L$ ) followed by a constant domain ( $C_L$ ) at its other end. The  $V_L$  is aligned with the  $V_H$  and the  $C_L$  is aligned with the first constant domain of the heavy chain ( $C_{H1}$ ). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a  $V_H$  and  $V_L$  together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains ( $C_H$ ), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$  and  $\mu$ , respectively. The  $\gamma$  and  $\alpha$  classes are further divided into subclasses on the basis of relatively minor differences in  $C_H$  sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and define specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a  $\beta$ -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the  $V_L$ , and around about 1-35 (H1), 50-65 (H2) and 95-102 (H3) in the  $V_H$ ; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the  $V_L$ , and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the  $V_H$ ; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal

antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present invention may be prepared by the hybridoma methodology first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example.

The monoclonal antibodies herein include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see U.S. Patent No. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (*e.g.* Old World Monkey, Ape etc), and human constant region sequences.

An "intact" antibody is one which comprises an antigen-binding site as well as a C<sub>L</sub> and at least heavy chain constant domains, C<sub>H</sub>1, C<sub>H</sub>2 and C<sub>H</sub>3. The constant domains may be native sequence constant domains (*e.g.* human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

An "antibody fragment" comprises a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies (see US patent 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V<sub>H</sub>), and the first constant domain of one heavy chain (C<sub>H</sub>1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')<sub>2</sub> fragment which roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having additional few residues at the carboxy terminus of the C<sub>H</sub>1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')<sub>2</sub> antibody fragments originally were produced as pairs of

Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, which region is also the part recognized by Fc receptors (FcR) found on certain types of cells.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

"Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the V<sub>H</sub> and V<sub>L</sub> antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); Borrebaeck 1995, *infra*.

The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10 residues) between the V<sub>H</sub> and V<sub>L</sub> domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, i.e., fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V<sub>H</sub> and V<sub>L</sub> domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (e.g., antibody) derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of a naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

The term "amino acid sequence variant" refers to a polypeptide that has amino acid sequences that differ to some extent from a native sequence polypeptide. Ordinarily, amino acid sequence variants of PSCA will possess at least about 70% homology with the native sequence PSCA, preferably, at least about 80%, more preferably at least about 85%, even more preferably at least about 90% homology, and most preferably at least 95%. The amino acid sequence variants can possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence.

The phrase "functional fragment or analog" of an antibody is a compound having qualitative biological activity in common with a full-length antibody. For example, a functional fragment or analog of an anti-IgE antibody is one which can bind to an IgE immunoglobulin in such a manner so as to prevent or

substantially reduce the ability of such molecule from having the ability to bind to the high affinity receptor, FcεRI.

“Homology” is defined as the percentage of residues in the amino acid sequence variant that are identical after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent  
5 homology. Methods and computer programs for the alignment are well known in the art. One such computer program is "Align 2", authored by Genentech, Inc., which was filed with user documentation in the United States Copyright Office, Washington, DC 20559, on December 10, 1991.

"Humanized" forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the most part, humanized antibodies are  
10 human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity, and capability. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not  
15 found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an  
20 immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

As used herein, an anti-PSCA antibody that “internalizes” is one that is taken up by (*i.e.*, enters) the cell upon binding to PSCA on a mammalian cell (*i.e.* cell surface PSCA). The internalizing antibody will of  
25 course include antibody fragments, human or humanized antibody and antibody conjugate. For therapeutic applications, internalization *in vivo* is contemplated. The number of antibody molecules internalized will be sufficient or adequate to kill a PSCA-expressing cell, especially a PSCA-expressing cancer cell. Depending on the potency of the antibody or antibody conjugate, in some instances, the uptake of a single antibody molecule into the cell is sufficient to kill the target cell to which the antibody binds. For example, certain  
30 toxins are highly potent in killing such that internalization of one molecule of the toxin conjugate to the antibody is sufficient to kill the tumor cell.

Whether an anti-PSCA antibody internalizes upon binding PSCA on a mammalian cell can be determined by various assays including those described in the experimental examples below. For example, to test internalization *in vivo*, the test antibody is labeled and introduced into an animal known to have PSCA  
35 expressed on the surface of certain cells. The antibody can be radiolabeled or labeled with fluorescent or gold particles, for instance. Animals suitable for this assay include a mammal such as a NCR nude mouse that contains a human PSCA-expressing tumor transplant or xenograft, or a mouse into which cells transfected with human PSCA have been introduced, or a transgenic mouse expressing the human PSCA transgene. Appropriate controls include animals that did not receive the test antibody or that received an  
40 unrelated antibody, and animals that received an antibody to another antigen on the cells of interest, which antibody is known to be internalized upon binding to the antigen (*e.g.*, HERCEPTIN which binds to Her2

expressed on the human breast tumor cell line, MCF-7 in Example 3). The antibody can be administered to the animal, e.g., by intravenous injection. At suitable time intervals, tissue sections of the animal can be prepared using known methods or as described in the experimental examples below, and analyzed by light microscopy or electron microscopy, for internalization as well as the location of the internalized antibody in the cell. For internalization *in vitro*, the cells can be incubated in tissue culture dishes in the presence or absence of the relevant antibodies added to the culture media and processed for microscopic analysis at desired time points. The presence of an internalized, labeled antibody in the cells can be directly visualized by microscopy or by autoradiography if radiolabeled antibody is used. Alternatively, in a quantitative biochemical assay, a population of cells comprising PSCA-expressing cells are contacted *in vitro* or *in vivo* with a radiolabeled test antibody and the cells (if contacted *in vivo*, cells are then isolated after a suitable amount of time) are treated with a protease or subjected to an acid wash to remove uninternalized antibody on the cell surface. The cells are ground up and the amount of protease resistant, radioactive counts per minute (cpm) associated with each batch of cells is measured by passing the homogenate through a scintillation counter. Based on the known specific activity of the radiolabeled antibody, the number of antibody molecules internalized per cell can be deduced from the scintillation counts of the ground-up cells. Cells are "contacted" with antibody *in vitro* preferably in solution form such as by adding the cells to the cell culture media in the culture dish or flask and mixing the antibody well with the media to ensure uniform exposure of the cells to the antibody. Instead of adding to the culture media, the cells can be contacted with the test antibody in an isotonic solution such as PBS in a test tube for the desired time period. *In vivo*, the cells are contacted with antibody by any suitable method of administering the test antibody such as the methods of administration described below when administered to a patient.

The faster the rate of internalization of the antibody upon binding to the PSCA expressing cell *in vivo*, the faster the desired killing or growth inhibitory effect on the target PSCA-expressing cell can be achieved, e.g., by a cytotoxic immunoconjugate. Preferably, the kinetics of internalization of the anti-PSCA antibodies are such that they favor rapid killing of the PSCA-expressing target cell. Therefore, it is desirable that the anti-PSCA antibody exhibit a rapid rate of internalization preferably, within 24 hours from administration of the antibody *in vivo*, more preferably within about 12 hours, even more preferably within about 30 minutes to 1 hour, and most preferably, within about 30 minutes. The present invention provides antibodies that internalize as fast as about 15 minutes from the time of introducing the anti-PSCA antibody *in vivo*. The antibody will preferably be internalized into the cell within a few hours upon binding to PSCA on the cell surface, preferably within 1 hour, even more preferably within 15-30 minutes.

To determine if a test antibody can compete for binding to the same epitope as the epitope bound by the anti-PSCA antibodies of the present invention including the antibodies produced by the hybridomas deposited with the ATCC, a cross-blocking assay e.g., a competitive ELISA assay can be performed. In an exemplary competitive ELISA assay, PSCA coated on the wells of a microtiter plate is pre-incubated with or without candidate competing antibody and then the biotin-labeled anti-PSCA antibody of the invention is added. The amount of labeled anti-PSCA antibody bound to the PSCA antigen in the wells is measured using avidin-peroxidase conjugate and appropriate substrate. The antibody can be labeled with a radioactive or fluorescent label or some other detectable and measurable label. The amount of labeled anti-PSCA antibody that bound to the antigen will have an indirect correlation to the ability of the candidate competing antibody (test antibody) to compete for binding to the same epitope, i.e., the greater the affinity of the test antibody for the same epitope, the

less labeled antibody will be bound to the antigen-coated wells. A candidate competing antibody is considered an antibody that binds substantially to the same epitope or that competes for binding to the same epitope as an anti-PSCA antibody of the invention if the candidate antibody can block binding of the PSCA antibody by at least 20%, preferably by at least 20-50%, even more preferably, by at least 50% as compared to the control performed  
5 in parallel in the absence of the candidate competing antibody (but may be in the presence of a known non-competing antibody). It will be understood that variations of this assay can be performed to arrive at the same quantitative value.

An antibody having a "biological characteristic" of a designated antibody, such as any of the monoclonal antibodies 10E3, 6F8, 8D11 and 5F2, is one which possesses one or more of the biological  
10 characteristics of that antibody which distinguish it from other antibodies that bind to the same antigen, PSCA. For example, an antibody with a biological characteristic of 6F8 will bind the same epitope as that bound by 6F8 (*e.g.* which competes for binding or blocks binding of monoclonal antibody 6F8 to PSCA), be able to target a PSCA expressing tumor cell *in vivo* and will internalize upon binding to PSCA on a mammalian cell *in vivo*. Likewise, an antibody with the biological characteristic of the 5F2 or 6B8 antibody  
15 will have the same epitope binding, targeting, internalizing, tumor growth inhibitory and cytotoxic properties of the antibody.

The term "antagonist" antibody is used in the broadest sense, and includes an antibody that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PSCA protein disclosed herein. Methods for identifying antagonists of a PSCA polypeptide may comprise contacting a PSCA polypeptide or  
20 a cell expressing PSCA on the cell surface, with a candidate antagonist antibody and measuring a detectable change in one or more biological activities normally associated with the PSCA polypeptide.

An "antibody that inhibits the growth of tumor cells expressing PSCA" or a "growth inhibitory" antibody is one which binds to and results in measurable growth inhibition of cancer cells expressing or overexpressing PSCA. Preferred growth inhibitory anti-PSCA antibodies inhibit growth of PSCA-expressing  
25 tumor cells (*e.g.*, prostate cancer cells) by greater than 20%, preferably from about 20% to about 50%, and even more preferably, by greater than 50% (*e.g.* from about 50% to about 100%) as compared to the appropriate control, the control typically being tumor cells not treated with the antibody being tested. Growth inhibition can be measured at an antibody concentration of about 0.1 to 30  $\mu\text{g/ml}$  or about 0.5 nM to 200 nM in cell culture, where the growth inhibition is determined 1-10 days after exposure of the tumor cells to the  
30 antibody. Growth inhibition of tumor cells *in vivo* can be determined in various ways such as is described in the Experimental Examples section below. The antibody is growth inhibitory *in vivo* if administration of the anti-PSCA antibody at about 1  $\mu\text{g/kg}$  to about 100 mg/kg body weight results in reduction in tumor size or tumor cell proliferation within about 5 days to 3 months from the first administration of the antibody, preferably within about 5 to 30 days.

An antibody which "induces apoptosis" is one which induces programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is usually one which overexpresses PSCA. Preferably the cell is a tumor cell, *e.g.* a prostate, breast, ovarian, stomach, endometrial, lung, kidney, colon, bladder cell. Various methods are available for evaluating the cellular  
40 events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by

annexin binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an annexin binding assay.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor); and B cell activation.

"Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.* Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998).

"Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see review M. in Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *J. Immunol.* 24:249 (1994)).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer



(NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source, *e.g.* from blood.

“Complement dependent cytotoxicity” or “CDC” refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.* as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996), may be performed.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (*e.g.* epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain, as well as head and neck cancer, and associated metastases.

A “PSCA-expressing cell” is a cell which expresses endogenous or transfected PSCA on the cell surface. A “PSCA-expressing cancer” is a cancer comprising cells that have PSCA protein present on the cell surface. A “PSCA-expressing cancer” produces sufficient levels of PSCA on the surface of cells thereof, such that an anti-PSCA antibody can bind thereto and have a therapeutic effect with respect to the cancer. A cancer which “overexpresses” PSCA is one which has significantly higher levels of PSCA at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. PSCA overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the PSCA protein present on the surface of a cell (*e.g.* via an immunohistochemistry assay; FACS analysis). Alternatively, or additionally, one may measure levels of PSCA-encoding nucleic acid or mRNA in the cell, *e.g.* via fluorescent *in situ* hybridization; (FISH; see WO98/45479 published October, 1998), Southern blotting, Northern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study PSCA overexpression by measuring shed antigen in a biological fluid such as serum, *e.g.* using antibody-based assays (see also, *e.g.*, U.S. Patent No. 4,933,294 issued June 12, 1990; WO91/05264 published April 18, 1991; U.S. Patent 5,401,638 issued March 28, 1995; and Sias *et al.* *J. Immunol. Methods* 132: 73-80 (1990)). Aside from the above assays, various *in vivo* assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.* a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.* by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody. A PSCA-expressing cancer includes prostate, bladder, lung, uterine and breast cancer.

A “mammal” for purposes of treating a cancer or alleviating the symptoms of cancer, refers to any mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

"Treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. A subject or mammal is successfully "treated" for a PSCA-expressing cancer if, after receiving a therapeutic amount of an anti-PSCA antibody according to the methods of the present invention, the patient shows observable and/or measurable reduction in or absence of one or more of the following: reduction of PSA levels, reduction in the number of cancer cells or absence of the cancer cells; reduction in the tumor size; inhibition (*i.e.*, slow to some extent and preferably stop) of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition (*i.e.*, slow to some extent and preferably stop) of tumor metastasis; inhibition, to some extent, of tumor growth; and/or relief to some extent, one or more of the symptoms associated with the specific cancer; reduced morbidity and mortality, and improvement in quality of life issues. To the extent the anti-PSCA antibody may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. Reduction of these signs or symptoms may also be felt by the patient.

The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician. For cancer therapy, efficacy can be measured, for example, by assessing the time to disease progression (TTP) and/or determining the response rate (RR). For prostate cancer, the progress of therapy can be assessed by routine methods, usually by measuring serum PSA (prostate specific antigen) levels; the higher the level of PSA in the blood, the more extensive the cancer. Commercial assays for detecting PSA are available, e.g., Hybitech Tandem-E and Tandem-R PSA assay kits, the Yang ProsCheck polyclonal assay (Yang Labs, Bellevue, WA), Abbott Imx (Abbott Labs, Abbott Park, IL), etc. Metastasis can be determined by staging tests and by bone scan and tests for calcium level and other enzymes to determine spread to the bone. CT scans can also be done to look for spread to the pelvis and lymph nodes in the area. Chest X-rays and measurement of liver enzyme levels by known methods are used to look for metastasis to the lungs and liver, respectively. Other routine methods for monitoring the disease include transrectal ultrasonography (TRUS) and transrectal needle biopsy (TRNB).

For bladder cancer, which is a more localized cancer, methods to determine progress of disease include urinary cytologic evaluation by cystoscopy, monitoring for presence of blood in the urine, visualization of the urothelial tract by sonography or an intravenous pyelogram, computed tomography (CT) and magnetic resonance imaging (MRI). The presence of distant metastases can be assessed by CT of the abdomen, chest x-rays, or radionuclide imaging of the skeleton.

The term "therapeutically effective amount" refers to an amount of an antibody or a drug effective to "treat" a disease or disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. See preceding definition of "treating". To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time.

"Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

5 "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum  
10 albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN<sup>TM</sup>, polyethylene glycol (PEG), and PLURONICS<sup>TM</sup>.

15 The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At<sup>211</sup>, I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup>, Re<sup>186</sup>, Re<sup>188</sup>, Sm<sup>153</sup>, Bi<sup>212</sup>, P<sup>32</sup> and radioactive isotopes of Lu), chemotherapeutic agents e.g. methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such  
20 as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits  
25 growth of a cell, especially a PSCA expressing cancer cell, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of PSCA expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin,  
30 daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc  
35 Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

"Label" as used herein refers to a detectable compound or composition which is conjugated directly  
40 or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g.

radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

The term "epitope tagged" used herein refers to a chimeric polypeptide comprising an anti-PSCA antibody polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the Ig polypeptide to which it is fused. The tag polypeptide is also preferably fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

10 A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

15 An "isolated nucleic acid" is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

20 "Vector" includes shuttle and expression vectors. Typically, the plasmid construct will also include an origin of replication (e.g., the ColE1 origin of replication) and a selectable marker (e.g., ampicillin or tetracycline resistance), for replication and selection, respectively, of the plasmids in bacteria. An "expression vector" refers to a vector that contains the necessary control sequences or regulatory elements for expression of the antibodies including antibody fragment of the invention, in bacterial or eukaryotic cells. 25 Suitable vectors are disclosed below.

The cell that produces an anti-PSCA antibody of the invention will include the parent hybridoma cell e.g., the hybridomas that are deposited with the ATCC, as well as bacterial and eukaryotic host cells into which nucleic acid encoding the antibodies have been introduced. Suitable host cells are disclosed below.

## 30 II. Compositions and Methods of the Invention

The invention provides anti-PSCA antibodies. In a preferred embodiment, the anti-PSCA antibodies internalize upon binding to cell surface PSCA on a mammalian cell. In another preferred embodiment, the anti-PSCA antibodies destroy or lead to the destruction of tumor cells bearing PSCA.

35 The pathway and kinetics of internalization of GPI anchored molecules has not been as well studied as the clathrin coated receptor-mediated internalization pathway. It was not apparent that PSCA was internalization-competent. In addition, the ability of an antibody to internalize depends on several factors including the affinity, avidity, and isotype of the antibody, and the epitope that it binds. We have demonstrated herein that the GPI-linked, cell surface PSCA is internalization competent upon binding by the anti-PSCA antibodies of the invention. Additionally, it was demonstrated that the anti-PSCA antibodies of 40 the present invention can specifically target PSCA-expressing tumor cells in vivo and inhibit or kill these cells. These in vivo tumor targeting, internalization and growth inhibitory properties of the anti-PSCA

antibodies make these antibodies very suitable for therapeutic uses, e.g., in the treatment of various cancers including prostate, urinary tract and lung cancer. Internalization of the anti-PSCA antibody is preferred, e.g., if the antibody or antibody conjugate has an intracellular site of action and if the cytotoxic agent conjugated to the antibody does not readily cross the plasma (e.g., the toxin, calicheamicin). Internalization is not  
5 necessary if the antibodies or the agent conjugated to the antibodies do not have intracellular sites of action, e.g., if the antibody can kill the tumor cell by ADCC or some other mechanism.

The anti-PSCA antibodies of the invention also have various non-therapeutic applications. In view of the fact that the use of PSA as a tool for screening or diagnosing prostate cancer is controversial, the anti-PSCA antibodies of the present invention can be useful for diagnosis and staging of PSCA-expressing  
10 cancers (e.g., in radioimaging). The antibodies are also useful for purification or immunoprecipitation of PSCA from cells, for detection and quantitation of PSCA in vitro, e.g. in an ELISA or a Western blot, to kill and eliminate PSCA-expressing cells from a population of mixed cells as a step in the purification of other cells.

The internalizing anti-PSCA antibodies of the invention can be in the different forms encompassed  
15 by the definition of "antibody" herein. Thus, the antibodies include full length or intact antibody, antibody fragments, native sequence antibody or amino acid variants, humanized, chimeric or fusion antibodies, immunoconjugates, and functional fragments thereof. In fusion antibodies an antibody sequence is fused to a heterologous polypeptide sequence. The antibodies can be modified in the Fc region to provide desired effector functions. As discussed in more detail in the sections below, with the appropriate Fc regions, the  
20 naked antibody bound on the cell surface can induce cytotoxicity, e.g., via antibody-dependent cellular cytotoxicity (ADCC) or by recruiting complement in complement dependent cytotoxicity, or some other mechanism. Alternatively, where it is desirable to eliminate or reduce effector function, so as to minimize side effects or therapeutic complications, certain other Fc regions may be used.

In one embodiment, the antibody competes for binding or bind substantially to, the same epitope as  
25 the antibodies of the invention. Antibodies having the biological characteristics of the present anti-PSCA antibodies of the invention are also contemplated, e.g., an anti-PSCA antibody which has the biological characteristics of a monoclonal antibody produced by the hybridomas accorded ATCC accession numbers PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, or PTA-2264, specifically including the in vivo tumor targeting, internalization and any cell proliferation inhibition or cytotoxic characteristics.

30 Specifically provided are anti-PSCA antibodies that bind to an epitope present in amino acids 21-40, or 41-60, or 61-80, or 81-100 of human PSCA, shown in SEQ ID NO.1.

Methods of producing the above antibodies are described in detail below.

The present anti-PSCA antibodies are useful for treating a PSCA-expressing cancer or alleviating one or more symptoms of the cancer in a mammal. Such a cancer includes prostate cancer, cancer of the  
35 urinary tract, lung cancer, breast cancer, colon cancer and ovarian cancer, more specifically, prostate adenocarcinoma, renal cell carcinomas, colorectal adenocarcinomas, lung adenocarcinomas, lung squamous cell carcinomas, and pleural mesothelioma. The cancers encompass metastatic cancers of any of the preceding, e.g., prostate cancer metastases. The antibody is able to bind to at least a portion of the cancer cells that express PSCA in the mammal and preferably is one that does not induce or that minimizes HAMA  
40 response. In a preferred embodiment, the antibody is effective to destroy or kill PSCA-expressing tumor cells or inhibit the growth of such tumor cells, in vitro or in vivo, upon binding to PSCA on the cell. Such an

antibody includes a naked anti-PSCA antibody (not conjugated to any agent). Naked anti-PSCA antibodies having tumor growth inhibition properties *in vivo* include the antibodies described in the Experimental Examples below. Naked antibodies that have cytotoxic or cell growth inhibition properties can be further harnessed with a cytotoxic agent to render them even more potent in tumor cell destruction. Cytotoxic properties can be conferred to an anti-PSCA antibody by, e.g., conjugating the antibody with a cytotoxic agent, to form an immunoconjugate as described below. The cytotoxic agent or a growth inhibitory agent is preferably a small molecule. Toxins such as calicheamicin or a maytansinoid and analogs or derivatives thereof, are preferable.

The invention provides a composition comprising an anti-PSCA antibody of the invention, and a carrier. For the purposes of treating cancer, compositions can be administered to the patient in need of such treatment, wherein the composition can comprise one or more anti-PSCA antibodies present as an immunoconjugate or as the naked antibody. In a further embodiment, the compositions can comprise these antibodies in combination with other therapeutic agents such as cytotoxic or growth inhibitory agents, including chemotherapeutic agents. The invention also provides formulations comprising an anti-PSCA antibody of the invention, and a carrier. In one embodiment, the formulation is a therapeutic formulation comprising a pharmaceutically acceptable carrier.

Another aspect of the invention is isolated nucleic acids encoding the internalizing anti-PSCA antibodies. Nucleic acids encoding both the H and L chains and especially the hypervariable region residues, chains which encode the native sequence antibody as well as variants, modifications and humanized versions of the antibody, are encompassed.

The invention also provides methods useful for treating a PSCA-expressing cancer or alleviating one or more symptoms of the cancer in a mammal, comprising administering a therapeutically effective amount of an internalizing anti-PSCA antibody to the mammal. The antibody therapeutic compositions can be administered short term (acute) or chronic, or intermittent as directed by physician. Also provided are methods of inhibiting the growth of, and killing a PSCA expressing cell.

Finally, the invention also provides kits and articles of manufacture comprising at least one internalizing anti-PSCA antibody. Kits containing anti-PSCA antibodies find use e.g., for PSCA cell killing assays, for purification or immunoprecipitation of PSCA from cells. For example, for isolation and purification of PSCA, the kit can contain an anti-PSCA antibody coupled to beads (e.g., sepharose beads). Kits can be provided which contain the antibodies for detection and quantitation of PSCA *in vitro*, e.g. in an ELISA or a Western blot. Such antibody useful for detection may be provided with a label such as a fluorescent or radiolabel.

### III. Production of anti-PSCA Antibodies

The following describes exemplary techniques for the production of the antibodies useful in the present invention. Some of these techniques are described further in Example 1 and Table 2A. The PSCA antigen to be used for production of antibodies may be, e.g., the full length polypeptide or a portion thereof, including a soluble form of PSCA lacking the GPI anchor sequence (which can be obtained by enzymatic cleavage with phosphatidylinositol phospholipases), or synthetic peptides to selected portions of the protein. Alternatively, cells expressing PSCA at their cell surface (e.g. CHO or NIH-3T3 cells transformed to overexpress PSCA; prostate or other PSCA-expressing tumor cell line), or membranes prepared from such

cells can be used to generate antibodies. The nucleotide and amino acid sequences of human and murine PSCA are available as provided above. PSCA can be produced recombinantly in and isolated from, bacterial or eukaryotic cells using standard recombinant DNA methodology. PSCA can be expressed as a tagged (e.g., epitope tag) or other fusion protein to facilitate isolation as well as identification in various assays.

- 5 Antibodies or binding proteins that bind to various tags and fusion sequences are available as elaborated below. Other forms of PSCA useful for generating antibodies will be apparent to those skilled in the art.

#### Tags

- Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., *Mol. Cell. Biol.*, 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., *Protein Engineering*, 3(6):547-553 (1990)]. The FLAG-peptide [Hopp et al., *BioTechnology*, 6:1204-1210 (1988)] is recognized by an anti-FLAG M2 monoclonal antibody (Eastman Kodak Co., New Haven, CT). Purification of a protein containing the FLAG peptide can be performed by immunoaffinity chromatography using an affinity matrix comprising the anti-FLAG M2 monoclonal antibody covalently attached to agarose (Eastman Kodak Co., New Haven, CT). Other tag polypeptides include the KT3 epitope peptide [Martin et al., *Science*, 255:192-194 (1992)]; an  $\alpha$ -tubulin epitope peptide [Skinner et al., *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

#### (i) Polyclonal antibodies

- Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen (especially when synthetic peptides are used) to a protein that is immunogenic in the species to be immunized. For example, the antigen can be conjugated to keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, e.g., maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride,  $\text{SOCl}_2$ , or  $\text{R}^1\text{N}=\text{C}=\text{NR}$ , where R and  $\text{R}^1$  are different alkyl groups.

- Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100  $\mu\text{g}$  or 5  $\mu\text{g}$  of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) *Monoclonal antibodies*

Monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

5 In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

10 The hybridoma cells thus prepared are seeded and grown in a suitable culture medium which medium preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells (also referred to as fusion partner). For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium),  
15 which substances prevent the growth of HGPRT-deficient cells.

Preferred fusion partner myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution  
20 Center, San Diego, California USA, and SP-2 and derivatives e.g., X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); and Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

25 Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard  
30 analysis described in Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the  
35 hybridoma cells may be grown *in vivo* as ascites tumors in an animal e.g. by i.p. injection of the cells into mice.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (e.g., using protein A or protein G-Sepharose) or ion-exchange chromatography,  
40 hydroxylapatite chromatography, gel electrophoresis, dialysis, etc.



DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA that encodes the antibody may be modified to produce chimeric or fusion antibody polypeptides, for example, by substituting human heavy chain and light chain constant domain ( $C_H$  and  $C_L$ ) sequences for the homologous murine sequences (U.S. Patent No. 4,816,567; and Morrison, *et al.*, *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by fusing the immunoglobulin coding sequence with all or part of the coding sequence for a non-immunoglobulin polypeptide (heterologous polypeptide). The non-immunoglobulin polypeptide sequences can substitute for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

### (iii) Humanized antibodies

Methods for humanizing non-human antibodies have been described in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature*, 321:522-525 (1986); Reichmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity and HAMA response (human anti-mouse antibody) when the antibody is intended for human therapeutic use. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human V domain sequence which is closest to that of the rodent is identified and the human framework region (FR) within it accepted for the humanized antibody (Sims *et al.*, *J. Immunol.*, 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al.*, *J. Immunol.*, 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high binding affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Various forms of a humanized anti-PSCA antibody are contemplated. For example, the humanized antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody may be an intact antibody, such as an intact IgG1 antibody.

#### (iv) Human antibodies

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region ( $J_H$ ) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggemann *et al.*, *Year in Immuno.*, 7:33 (1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669 (all of GenPharm); 5,545,807; and WO 97/17852.

Alternatively, phage display technology (McCafferty *et al.*, *Nature* 348:552-553 [1990]) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain

gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats, reviewed in, *e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). See, also, U.S. Patent Nos. 5,565,332 and 5,573,905.

As discussed above, human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275).

(v) *Antibody fragments*

In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan *et al.*, *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')<sub>2</sub> fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')<sub>2</sub> fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')<sub>2</sub> fragment with increased *in vivo* half-life comprising a salvage receptor binding epitope residues are described in U.S. Patent No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Patent No. 5,571,894; and U.S. Patent No. 5,587,458. Fv and sFv are the only species with intact combining sites that are devoid of constant regions; thus, they are suitable for reduced nonspecific binding during *in vivo* use. sFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an sFv. See Antibody Engineering, ed. Borrebaeck, *supra*. The antibody fragment may also be a "linear antibody", *e.g.*, as described in U.S. Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) *Bispecific antibodies*

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the PSCA protein. Other such antibodies may combine an PSCA binding site with a binding site for another protein. Alternatively, an anti-  
5 PSCA arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16), so as to focus and localize cellular defense mechanisms to the PSCA-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express PSCA. These antibodies possess a PSCA-binding arm and an arm which binds the cytotoxic agent (*e.g.* saporin, anti-  
10 interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* F(ab')<sub>2</sub> bispecific antibodies).

WO 96/16673 describes a bispecific anti-ErbB2/anti-FcγRIII antibody and U.S. Patent No. 5,837,234 discloses a bispecific anti-ErbB2/anti-FcγRI antibody. A bispecific anti-ErbB2/Fcα antibody is shown in WO98/02463. U.S. Patent No. 5,821,337 teaches a bispecific anti-ErbB2/anti-CD3 antibody.

15 Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure.  
20 Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. Preferably, the  
25 fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, C<sub>H</sub>2, and C<sub>H</sub>3 regions. It is preferred to have the first heavy-chain constant region (C<sub>H</sub>1) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual  
30 proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant affect on the yield of the desired chain combination.

35 In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of  
40 the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690.

For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

According to another approach described in U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C<sub>H</sub>3 domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science*, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub> fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.*, 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a

V<sub>H</sub> connected to a V<sub>L</sub> by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V<sub>H</sub> and V<sub>L</sub> domains of one fragment are forced to pair with the complementary V<sub>L</sub> and V<sub>H</sub> domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.*, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.* *J. Immunol.* 147: 60 (1991).

(vii) *Multivalent Antibodies*

A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)<sub>n</sub>-VD2-(X2)<sub>n</sub>-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

(viii) *Other amino acid sequence modifications*

Amino acid sequence modification(s) of the anti-PSCA antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the anti-PSCA antibody are prepared by introducing appropriate nucleotide changes into the anti-PSCA antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the anti-PSCA antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the anti-PSCA antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the anti-PSCA antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells in *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with PSCA antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed anti-PSCA antibody variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an anti-PSCA antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the anti-PSCA antibody molecule include the fusion to the N- or C-terminus of the anti-PSCA antibody to an enzyme (*e.g.* for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the anti-PSCA antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

TABLE 1 Amino Acid Substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile

Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the anti-PSCA antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a



crystal structure of the antigen-antibody complex to identify contact points between the antibody and human PSCA. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Nucleic acid molecules encoding amino acid sequence variants of the anti-PSCA antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the anti-PSCA antibody.

It may be desirable to modify the antibody of the invention with respect to effector function, *e.g.* so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp. Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al. Anti-Cancer Drug Design* 3:219-230 (1989).

To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of

an IgG molecule (*e.g.*, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, or IgG<sub>4</sub>) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

(ix) *Screening for antibodies with the desired properties*

5 Techniques for generating antibodies have been described above. One may further select antibodies with certain biological characteristics, as desired.

The growth inhibitory effects of an anti-PSCA antibody of the invention may be assessed by methods known in the art, *e.g.*, using cells which express PSCA either endogenously or following transfection with the PSCA gene. For example, the tumor cell lines and PSCA-transfected cells provided in  
10 Example 2 below may be treated with an anti-PSCA monoclonal antibody of the invention at various concentrations for a few days (*e.g.*, 2-7) days and stained with crystal violet or MTT or analyzed by some other colorimetric assay. Another method of measuring proliferation would be by comparing <sup>3</sup>H-thymidine uptake by the cells treated in the presence or absence of an anti-PSCA antibody of the invention. After antibody treatment, the cells are harvested and the amount of radioactivity incorporated into the DNA quantitated in a  
15 scintillation counter. Appropriate positive controls include treatment of a selected cell line with a growth inhibitory antibody known to inhibit growth of that cell line. Growth inhibition of tumor cells *in vivo* can be determined in various ways such as is described in the Experimental Examples section below (see Example 6). Preferably, the tumor cell is one that over-expresses PSCA. Preferably, the anti-PSCA antibody will inhibit cell proliferation of a PSCA-expressing tumor cell *in vitro* or *in vivo* by about 25-100% compared to  
20 the untreated tumor cell, more preferably, by about 30-100%, and even more preferably by about 50-100% or 70-100%, at an antibody concentration of about 0.5 to 30 µg/ml. Growth inhibition can be measured at an antibody concentration of about 0.5 to 30 µg/ml or about 0.5 nM to 200 nM in cell culture, where the growth inhibition is determined 1-10 days after exposure of the tumor cells to the antibody. The antibody is growth inhibitory *in vivo* if administration of the anti-PSCA antibody at about 1 µg/kg to about 100 mg/kg body  
25 weight results in reduction in tumor size or tumor cell proliferation within about 5 days to 3 months from the first administration of the antibody, preferably within about 5 to 30 days.

To select for antibodies which induce cell death, loss of membrane integrity as indicated by, *e.g.*, propidium iodide (PI), trypan blue or 7AAD uptake may be assessed relative to control. A PI uptake assay can be performed in the absence of complement and immune effector cells. PSCA-expressing tumor cells are  
30 incubated with medium alone or medium containing the appropriate monoclonal antibody at *e.g.*, about 10 µg/ml. The cells are incubated for a 3 day time period. Following each treatment, cells are washed and aliquoted into 35 mm strainer-capped 12 x 75 tubes (1ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 µg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce  
35 statistically significant levels of cell death as determined by PI uptake may be selected as cell death-inducing antibodies.

To screen for antibodies which bind to an epitope on PSCA bound by an antibody of interest, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. This assay can be used to determine if a  
40 test antibody binds the same site or epitope as an anti-PSCA antibody of the invention. Alternatively, or

additionally, epitope mapping can be performed by methods known in the art. For example, the antibody sequence can be mutagenized such as by alanine scanning, to identify contact residues. The mutant antibody is initially tested for binding with polyclonal antibody to ensure proper folding. In a different method, peptides corresponding to different regions of PSCA can be used in competition assays with the test antibodies or with a test antibody and an antibody with a characterized or known epitope.

(x) *Immunoconjugates*

The invention also pertains to therapy with immunoconjugates comprising an antibody conjugated to an anti-cancer agent such as a cytotoxic agent or a growth inhibitory agent.

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above.

Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, maytansinoids, a trichothene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein.

A. Maytansine and maytansinoids

In one preferred embodiment, an anti-PSCA antibody (full length or fragments) of the invention is conjugated to one or more maytansinoid molecules.

Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Patent No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533, the disclosures of which are hereby expressly incorporated by reference.

B. Maytansinoid-antibody conjugates

In an attempt to improve their therapeutic index, maytansine and maytansinoids have been conjugated to antibodies specifically binding to tumor cell antigens. Immunoconjugates containing maytansinoids and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. Liu *et al.*, Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described immunoconjugates comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an *in vivo* tumor growth assay. Chari *et al.* Cancer Research 52:127-131 (1992) describe immunoconjugates in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/*neu* oncogene. The cytotoxicity of the TA.1-maytansinoid conjugate was tested *in vitro* on the human breast cancer cell line SK-BR-3, which expresses  $3 \times 10^5$  HER-2 surface

antigens per cell. The drug conjugate achieved a degree of cytotoxicity similar to the free maytansinoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

5 C. Anti-PSCA antibody-maytansinoid conjugates (immunoconjugates)

Anti-PSCA antibody-maytansinoid conjugates are prepared by chemically linking an anti-PSCA antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. An average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody, although even one molecule of toxin/antibody would be expected to enhance cytotoxicity over the use of naked antibody. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed, for example, in U.S. Patent No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytansinoids are maytansinol and maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

There are many linking groups known in the art for making antibody-maytansinoid conjugates, including, for example, those disclosed in U.S. Patent No. 5,208,020 or EP Patent 0 425 235 B1, and Chari *et al. Cancer Research* 52: 127-131 (1992). The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents, disulfide and thioether groups being preferred.

Conjugates of the antibody and maytansinoid may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particularly preferred coupling agents include N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (Carlsson *et al., Biochem. J.* 173:723-737 [1978]) and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage. See Example 7 below.

The linker may be attached to the maytansinoid molecule at various positions, depending on the type of the link. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. The reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In a preferred embodiment, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

Calicheamicin

Another immunoconjugate of interest comprises an anti-PSCA antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. For the preparation of conjugates of the calicheamicin family,

see U.S. patents 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, 5,877,296 (all to American Cyanamid Company). Structural analogues of calicheamicin which may be used include, but are not limited to,  $\gamma_1^I$ ,  $\alpha_2^I$ ,  $\alpha_3^I$ , N-acetyl- $\gamma_1^I$ , PSAG and  $\theta_1^I$  (Hinman *et al. Cancer Research* 53: 3336-3342 (1993), Lode *et al. Cancer Research* 58: 2925-2928 (1998) and the aforementioned U.S. patents to American Cyanamid). Another anti-tumor drug that the antibody can be conjugated is QFA which is an antifolate. Both calicheamicin and QFA have intracellular sites of action and do not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody mediated internalization greatly enhances their cytotoxic effects.

#### 10      Other cytotoxic agents

Other antitumor agents that can be conjugated to the anti-PSCA antibodies of the invention include BCNU, streptozocin, vincristine and 5-fluorouracil, the family of agents known collectively LL-E33288 complex described in U.S. patents 5,053,394, 5,770,710, as well as esperamicins (U.S. patent 5,877,296).

15      Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

20      The present invention further contemplates an immunoconjugate formed between an antibody and a compound with nucleolytic activity (*e.g.* a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

For selective destruction of the tumor, the antibody may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated anti-PSCA antibodies. Examples include  $At^{211}$ ,  $I^{131}$ ,  $I^{125}$ ,  $Y^{90}$ ,  $Re^{186}$ ,  $Re^{188}$ ,  $Sm^{153}$ ,  $Bi^{212}$ ,  $P^{32}$ ,  $Pb^{212}$  and radioactive isotopes of Lu. When the conjugate is used for diagnosis, it may comprise a radioactive atom for scintigraphic studies, for example  $tc^{99m}$  or  $I^{123}$ , or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

30      The radio- or other labels may be incorporated in the conjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as  $tc^{99m}$  or  $I^{123}$ ,  $Re^{186}$ ,  $Re^{188}$  and  $In^{111}$  can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker *et al* (1978) *Biochem. Biophys. Res. Commun.* 80: 49-57 can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunosciintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

40      Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as

glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-  
5 labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari *et al. Cancer Research* 52: 127-131 (1992); U.S. Patent No. 5,208,020) may be used.

10 Alternatively, a fusion protein comprising the anti-PSCA antibody and cytotoxic agent may be made, *e.g.* by recombinant techniques or peptide synthesis. The length of DNA may comprise respective regions encoding the two portions of the conjugate either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for  
15 utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.* avidin) which is conjugated to a cytotoxic agent (*e.g.* a radionucleotide).

(xi) *Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT)*

20 The antibodies of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of  
25 acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting  
30 peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as  $\beta$ -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs;  $\beta$ -lactamase useful for converting drugs derivatized with  $\beta$ -lactams into free drugs; and penicillin amidases, such as penicillin V amidase or  
35 penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the anti-PSCA antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g., Neuberger *et al.*, *Nature*, 312: 604-608 (1984).

(xi) *Other antibody modifications*

Other modifications of the antibody are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, 16th edition, Oslo, A., Ed., (1980).

The anti-PSCA antibodies disclosed herein may also be formulated as immunoliposomes. A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.* *J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon *et al.* *J. National Cancer Inst.* 81(19)1484 (1989).

#### IV. Vectors, Host Cells and Recombinant Methods

The invention also provides isolated nucleic acid encoding the humanized anti-PSCA antibody, vectors and host cells comprising the nucleic acid, and recombinant techniques for the production of the antibody.

For recombinant production of the antibody, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(i) *Signal sequence component*

The anti-PSCA antibody of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native anti-PSCA antibody signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader,  $\alpha$  factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

The DNA for such precursor region is ligated in reading frame to DNA encoding the anti-PSCA antibody.

(ii) *Origin of replication*

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 $\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

(iii) *Selection gene component*

Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the anti-PSCA antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, *etc.*



For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL-9096).

5 Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding anti-PSCA antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

10 A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, *Nature*, 282:39 (1979)). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics*, 85:12 (1977). The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient  
15 yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

In addition, vectors derived from the 1.6  $\mu$ m circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Alternatively, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg, *Bio/Technology*, 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of  
20 *Kluyveromyces* have also been disclosed. Fleer *et al.*, *Bio/Technology*, 9:968-975 (1991).

#### (iv) Promoter component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the anti-PSCA antibody nucleic acid. Promoters suitable for use with prokaryotic  
25 hosts include the *phoA* promoter,  $\beta$ -lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan (*trp*) promoter system, and hybrid promoters such as the *tac* promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the anti-PSCA antibody.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich  
30 region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

35 Examples of suitable promoter sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

40 Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2,

isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

5 Anti-PSCA antibody transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are  
10 compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446.  
15 A modification of this system is described in U.S. Patent No. 4,601,978. See also Reyes *et al.*, *Nature* 297:598-601 (1982) on expression of human  $\beta$ -interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

20 (v) *Enhancer element component*

Transcription of a DNA encoding the anti-PSCA antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the  
25 replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the anti-PSCA antibody-encoding sequence, but is preferably located at a site 5' from the promoter.

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(vi) *Transcription termination component*

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and,  
35 occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding anti-PSCA antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

(vii) *Selection and transformation of host cells*

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

Full length antibody, antibody fragments, and antibody fusion proteins can be produced in bacteria, in particular when glycosylation and Fc effector function are not needed, such as when the therapeutic antibody is conjugated to a cytotoxic agent (e.g., a toxin) and the immunoconjugate by itself shows effectiveness in tumor cell destruction. Full length antibodies have greater half life in circulation. Production in *E. coli* is faster and more cost efficient. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. 5,648,237 (Carter et. al.), U.S. 5,789,199 (Joly et al.), and U.S. 5,840,523 (Simmons et al.) which describes translation initiation regio (TIR) and signal sequences for optimizing expression and secretion, these patents incorporated herein by reference. After expression, the antibody is isolated from the *E. coli* cell paste in a soluble fraction and can be purified through, e.g., a protein A or G column depending on the isotype. Final purification can be carried out similar to the process for purifying antibody expressed e.g., in CHO cells.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for anti-PSCA antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, e.g., *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

Suitable host cells for the expression of glycosylated anti-PSCA antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)) ; baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)) ; mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)) ; monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors for anti-PSCA antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

15

(viii) *Culturing the host cells*

The host cells used to produce the anti-PSCA antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM), Sigma are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN<sup>TM</sup> drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

30

(ix) *Purification of anti-PSCA antibody*

When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF

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may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human  $\gamma 1$ ,  $\gamma 2$ , or  $\gamma 4$  heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human  $\gamma 3$  (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a  $C_H3$  domain, the Bakerbond ABX<sup>TM</sup>resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE<sup>TM</sup> chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

## V. Pharmaceutical Formulations

Therapeutic formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as acetate, Tris, phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; tonicifiers such as trehalose and sodium chloride; sugars such as sucrose, mannitol, trehalose or sorbitol; surfactant such as polysorbate; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN<sup>TM</sup>, PLURONICS<sup>TM</sup> or polyethylene glycol (PEG). The antibody preferably comprises the antibody at a concentration of between 5-200 mg/ml, preferably between 10-100 mg/ml.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, in addition to the anti-PSCA antibody which internalizes, it may be desirable to include in the one formulation, an additional antibody, *e.g.* a second anti-PSCA antibody which binds a different epitope on PSCA, or an antibody to some other target such as a growth factor that affects the growth of the particular cancer. Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

## VI. Treatment with the Anti-PSCA Antibodies

According to the present invention, the anti-PSCA antibody that internalizes upon binding PSCA on a cell surface is used to treat a PSCA-expressing cancer cell, in particular, bladder and prostate cancer, such as androgen independent prostate cancer or androgen dependent prostate cancer, and associated metastases. A patient may be diagnosed as having androgen independent prostate cancer in that he no longer responds to anti-androgen therapy and the patient diagnosed as having androgen dependent prostate cancer may be one who responds to anti-androgen therapy. The cancer will generally comprise PSCA-expressing cells, such that the anti-PSCA antibody is able to bind thereto. While the cancer may be characterized by overexpression of the PSCA molecule, the present application further provides a method for treating cancer which is not considered to be an PSCA-overexpressing cancer.

To determine PSCA expression in the cancer, various diagnostic assays are available. In one embodiment, PSCA overexpression may be analyzed by immunohistochemistry (IHC). Paraffin embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a PSCA protein staining intensity criteria as follows:

Score 0 no staining is observed or membrane staining is observed in less than 10% of tumor cells.

Score 1+ a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.

5 Score 2+ a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

Score 3+ a moderate to strong complete membrane staining is observed in more than 10% of the tumor cells.

Those tumors with 0 or 1+ scores for PSCA expression may be characterized as not overexpressing PSCA, whereas those tumors with 2+ or 3+ scores may be characterized as overexpressing PSCA.

Alternatively, or additionally, FISH assays such as the INFORM™ (sold by Ventana, Arizona) or  
10 PATHVISION™ (Vysis, Illinois) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of PSCA overexpression in the tumor.

PSCA overexpression or amplification may be evaluated using an *in vivo* diagnostic assay, *e.g.* by administering a molecule (such as an antibody) which binds the molecule to be detected and is tagged with a detectable label (*e.g.* a radioactive isotope or a fluorescent label) and externally scanning the patient for  
15 localization of the label.

Currently, depending on the stage of the cancer, prostate cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, androgen deprivation (*e.g.*, hormonal therapy), and chemotherapy. Anti-PSCA antibody therapy may be especially desirable in elderly patients who do not tolerate the toxicity and side effects of chemotherapy well, in  
20 metastatic disease where radiation therapy has limited usefulness, and for the management of prostatic carcinoma that is resistant to androgen deprivation treatment. The tumor targeting and internalizing anti-PSCA antibodies of the invention are useful to alleviate PSCA-expressing cancers, *e.g.* prostate and bladder cancers upon initial diagnosis of the disease or during relapse. For therapeutic applications, the anti-PSCA antibody can be used alone, or in combination therapy with, *e.g.*, hormones, antiangiogens, or radiolabelled  
25 compounds, or with surgery, cryotherapy, and/or radiotherapy, notably for prostate cancers, also particularly where shed cells cannot be reached. Anti-PSCA antibody treatment can be administered in conjunction with other forms of conventional therapy, either consecutively with, pre- or post-conventional therapy. Chemotherapeutic drugs such as taxotere® (docetaxel), taxol® (paclitaxel), estramustine and mitoxantrone are used in treating metastatic and hormone refractory prostate cancer, in particular, in good risk patients. In  
30 the present method of the invention for treating or alleviating cancer, in particular, androgen independent and/or metastatic prostate cancer, the cancer patient can be administered anti-PSCA antibody in conjunction with treatment with the one or more of the preceding chemotherapeutic agents. In particular, combination therapy with paclitaxel and modified derivatives (see, *e.g.*, EP0600517) is contemplated. The anti-PSCA antibody will be administered with a therapeutically effective dose of the chemotherapeutic agent. In another  
35 embodiment, the anti-PSCA antibody is administered in conjunction with chemotherapy to enhance the activity and efficacy of the chemotherapeutic agent, *e.g.*, paclitaxel. The Physicians' Desk Reference (PDR) discloses dosages of these agents that have been used in treatment of various cancers. The dosing regimen and dosages of these aforementioned chemotherapeutic drugs that are therapeutically effective will depend on the particular cancer being treated, the extent of the disease and other factors familiar to the physician of skill  
40 in the art and can be determined by the physician.

In one particular embodiment, an immunoconjugate comprising the anti-PSCA antibody conjugated with a cytotoxic agent is administered to the patient. Preferably, the immunoconjugate bound to the PSCA protein is internalized by the cell, resulting in increased therapeutic efficacy of the immunoconjugate in killing the cancer cell to which it binds. In a preferred embodiment, the cytotoxic agent targets or interferes with the nucleic acid in the cancer cell. Examples of such cytotoxic agents are described above and include maytansinoids, calicheamicins, ribonucleases and DNA endonucleases.

The anti-PSCA antibodies or immunoconjugates are administered to a human patient, in accord with known methods, such as intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the antibody is preferred.

Other therapeutic regimens may be combined with the administration of the anti-PSCA antibody. The combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Preferably such combined therapy results in a synergistic therapeutic effect.

It may also be desirable to combine administration of the anti-PSCA antibody or antibodies, with administration of an antibody directed against another tumor antigen associated with the particular cancer.

In another embodiment, the antibody therapeutic treatment method of the present invention involves the combined administration of an anti-PSCA antibody (or antibodies) and one or more chemotherapeutic agents or growth inhibitory agents, including co-administration of cocktails of different chemotherapeutic agents. Chemotherapeutic agents include estramustine phosphate, prednimustine, cisplatin, 5-fluorouracil, melphalan, cyclophosphamide, hydroxyurea and hydroxyureataxanes (such as paclitaxel and doxorubicin) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992).

The antibody may be combined with an anti-hormonal compound; *e.g.*, an anti-estrogen compound such as tamoxifen; an anti-progesterone such as onapristone (see, EP 616 812); or an anti-androgen such as flutamide, in dosages known for such molecules. Where the cancer to be treated is androgen independent cancer, the patient may previously have been subjected to anti-androgen therapy and, after the cancer becomes androgen independent, the anti-PSCA antibody (and optionally other agents as described herein) may be administered to the patient.

Sometimes, it may be beneficial to also co-administer a cardioprotectant (to prevent or reduce myocardial dysfunction associated with the therapy) or one or more cytokines to the patient. In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy, before, simultaneously with, or post antibody therapy. Suitable dosages for any of the above co-administered agents are those presently used and may be lowered due to the combined action (synergy) of the agent and anti-PSCA antibody.

For the prevention or treatment of disease, the dosage and mode of administration will be chosen by the physician according to known criteria. The appropriate dosage of antibody will depend on the type of



disease to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Preferably, the antibody is administered by intravenous infusion or by subcutaneous injections. Depending on the type and severity of the disease, about 1 µg/kg to about 50 mg/kg body weight (*e.g.* about 0.1-15mg/kg/dose) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A dosing regimen can comprise administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the anti-PSCA antibody. However, other dosage regimens may be useful. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. The progress of this therapy can be readily monitored by conventional methods and assays and based on criteria known to the physician or other persons of skill in the art.

Aside from administration of the antibody protein to the patient, the present application contemplates administration of the antibody by gene therapy. Such administration of nucleic acid encoding the antibody is encompassed by the expression "administering a therapeutically effective amount of an antibody". See, for example, WO96/07321 published March 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells; *in vivo* and *ex vivo*. For *in vivo* delivery the nucleic acid is injected directly into the patient, usually at the site where the antibody is required. For *ex vivo* treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes which are implanted into the patient (see, *e.g.* U.S. Patent Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. A commonly used vector for *ex vivo* delivery of the gene is a retroviral vector.

The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). For review of the currently known gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256:808-813 (1992). See also WO 93/25673 and the references cited therein.

## VII. Articles of Manufacture and Kits

Another embodiment of the invention is an article of manufacture containing materials useful for the treatment of anti-PSCA expressing cancer, in particular prostate cancer and bladder cancer. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of

materials such as glass or plastic. The container holds a composition which is effective for treating the cancer condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-PSCA antibody of the invention. The label or package insert indicates that the composition is used for treating prostate cancer, androgen independent prostate cancer, or androgen dependent prostate cancer, or bladder cancer. The label or package insert will further comprise instructions for administering the antibody composition to the cancer patient. Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Kits are also provided that are useful for various purposes, e.g., for PSCA cell killing assays, for purification or immunoprecipitation of PSCA from cells. For isolation and purification of PSCA, the kit can contain an anti-PSCA antibody coupled to beads (e.g., sepharose beads). Kits can be provided which contain the antibodies for detection and quantitation of PSCA *in vitro*, e.g. in an ELISA or a Western blot. As with the article of manufacture, the kit comprises a container and a label or package insert on or associated with the container. The container holds a composition comprising at least one anti-PSCA antibody of the invention. Additional containers may be included that contain, e.g., diluents and buffers, control antibodies. The label or package insert may provide a description of the composition as well as instructions for the intended *in vitro* or diagnostic use.

### VIII. Experimental Examples

The above and other features of the invention will now be described more particularly with reference to the accompanying figures and pointed out in the claims. The particular embodiments described below are provided by way of illustration and are not meant to be construed as a limitation on the scope of the invention. It will be apparent to one of ordinary skill in the art that many modifications can be made to the present invention without departing from the spirit or essential characteristics of the invention.

#### Example 1

The preparation and characterization of the anti-PSCA monoclonal antibodies are described.

##### I. Materials and Methods

Hybridomas producing murine and human anti-PSCA monoclonal antibodies (Mabs) were generated. The fusion partner was the mouse myeloma line P3X63Ag8.653 (Kearney, J.F. et al., J. Immunology 123:1548-1550, 1979).

Table 2A summarizes the immunization strategies and shows the nature of the antigen used for immunization, the adjuvant, dosage, route of administration, dosing schedule and host animal used. Table 2B summarizes the characteristics of the anti-PSCA Mabs as determined by various assays. The antibody names herein are derived from the first 3-4 characters of the hybridoma clone producing the antibody (see Table 2B). The first 3-4 characters before the period in the clone name indicate the parental clone followed after the first period by the name of further subclones, if any. Alternatively, the anti-PSCA antibodies herein are also referred to by their designated ascites number. Except where muPSCA is indicated, human (hu) PSCA

by itself or fused to a gD tag (gDPSCA) or histidine tag (his-PSCA), was used. Human or murine PSCA expressed in E.coli was histidine-tagged. Unless otherwise indicated, PSCA expressed in E.coli was refolded, as described below, for immunization. gDPSCA-CHO refers to soluble PSCA expressed in and purified from CHO cells. Antibodies prepared from Xenomice (Abgenix, Fremont, California) are fully human antibodies.

For the hu PSCA nucleotide and amino acid sequences, refer to WO 99/14328 (Genentech; PRO232 protein, nucleotide sequence in Fig. 1; amino acid sequence in Fig. 2); U.S. Patent 5,856,136 issued January 5, 1999 (Incyte; SCAH2 SEQ ID4); or Fig. 2 on page 1737 of Reiter et al. *Proc. Nat. Acad. Sci.* 95:1735-1740 (1998). For convenience, the 123 amino acid sequence and the coding sequence of the cDNA of PSCA are provided herein as SEQ ID NO. 1 and SEQ ID NO. 2, respectively. The numbering of the amino acid sequence referred to herein will be according to that shown in SEQ ID NO. 1 which is the same as in Reiter et al. (1998).

### Cell Lines and Transfections

A plasmid vector encoding hu PSCA or gDPSCA was transfected into the following cells: NIH 3T3 cells expressing Ras (Ras 3T3); and CHO; HCT116. For expression in E. coli, the sequence encoding hu PSCA amino acids 10-101 was used (fused to a His tag), with the GPI anchor sequence removed. For expression in 3T3 cells, the N-terminal signal sequence (aa 1-20) of PSCA was replaced by the signal sequence of the gD tag followed by 25 amino acids of the mature gD.

### Binding Assays

The anti-PSCA antibodies were analyzed for binding to PSCA on cells by Fluorescence Activated Cell Sorter (FACS). Purified murine anti-PSCA 6B8.1D7.2B3 (#2761) antibody and fully human anti-PSCA 10C5.6E4.6D1 (#2910) antibody from hybridoma supernatant were analyzed for cell surface binding by FACS. Confluent adherent stable CHO cells transfected with huPSCA with a gD tag at its N-terminus were washed with PBS and detached using Cell Dissociation Solution (Sigma). Cells were incubated with the anti-PSCA antibodies at various concentrations (1-10  $\mu$ g/ml) and washed with PBS prior to incubation with a FITC conjugated secondary antibody. Cells were resuspended in PBS containing 1% FBS or 1% formaldehyde for cell fixation. The binding of antibodies to the cell surface was then analyzed on a FACScan.

To assess the ability of the Mabs to capture PSCA in solution, an assay was developed in which each antibody was either directly coated onto microtiter plates or bound to an anti-mouse IgG specific to the Fc region, previously coated on the plate. Biotinylated PSCA antigen (1  $\mu$ g/ml) was then added to the plate prior to incubation with streptavidin-HRP. OPD was used as a substrate and plates were read at 492nm.

To verify the binding of Mab #2403 to PSCA expressed on the surface of transfected cells, a cold competition binding assay was performed using  $^{125}$ I-labeled Mab #2403 (lactoperoxidase method). PC3.gD.hu PSCA cells ( $1 \times 10^5$ ) were plated in a 24 well dish and incubated in medium with various concentrations of unlabeled Mab 2403 and a constant amount of  $^{125}$ I-Mab 2403 for 16 hours at 4°C. Unbound antibody was removed and cells were washed with ice-cold medium. After solubilization of the cells in 8M Urea/3M glacial acetic acid, the amount of radioactivity bound was determined using a gamma

counter. The IC<sub>50</sub> value was calculated from a four parameter fit calculation of the curve where m3 represent the IC<sub>50</sub>. The results of this assay are plotted in Figure 4.

A direct ELISA was performed to determine the binding activity of the #2761 (6B8) antibody. HuPSCA and murine PSCA (mPSCA) were coated overnight at 1µg/ml onto a microtiter plate (Nunc). The anti-PSCA Mab and controls were added to the antigen-coated wells and incubated at room temperature. Unbound antibody was washed off and a horseradish peroxidase (HRP)-conjugated secondary antibody was added to the wells and incubated with the antibodies at room temperature. OPD substrate solution was finally added and concentrated sulfuric acid was used to stop the reaction. The plate was read at 492 nm using a microtiter plate reader.

Epitope groupings were determined by competitive ELISA. Each antibody was biotinylated and tested for binding to PSCA coated on plates, in the presence or absence of an excess of each unlabeled anti-PSCA Mab. Streptavidin-HRP was then added to the plates followed by peroxidase substrate. Decrease (at least 50%) or lack of binding of biotinylated Mabs to PSCA indicated that both cold and biotinylated antibodies bound to the same (or proximal) epitope on PSCA.

#### **Purification and refolding of His-tagged proteins produced in *E. coli***

The following procedure has been adapted for use on proteins produced from 0.5 to 1L fermentations of *E. coli* expressing His-tagged proteins. It can easily be scaled to much larger amounts.

*E. coli* paste from 0.5 to 1L fermentations ( 6-10 gm pellets) was resuspended in 10 volumes (w/v) in 7M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate were added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution was stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution was centrifuged at 40 K rpm in a Beckman Ultracentrifuge for 30 min. The supernatant was diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. Depending on the expression of the desired protein, 30-50 mls of the clarified extract were loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column was washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein was eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein were pooled and stored at 4°C. Protein concentration was estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins are usually refolded by diluting sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. In the case of PSCA, 3.5M Urea and 1 mM cysteine were used instead of 2.5 M Urea and 5 mM cysteine. Refolding volumes were chosen so that the final protein concentration would be between 50 to 100 micrograms/ml. The refolding solution was stirred gently at 4°C for 12-36 hours. The refolding reaction was quenched by the addition of trifluoroacetic acid (TFA) to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution was filtered through a 0.22 micron filter and acetonitrile added to 2-10% final concentration. The refolded protein was chromatographed on a Vydac C4 reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10% to 80%. Aliquots of fractions with A280 absorbance were analyzed on SDS polyacrylamide gels and

fractions containing homogeneous refolded protein were pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded protein were pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins were usually formulated into 20 mM Hepes, pH 6.8 with 0.14 M NaCl and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered. Occasionally, proteins with isoelectric points in the pH range of 6-7.5 would be formulated in a buffer of 1 mM HCl, 0.14 M NaCl to maintain solubility. Formulated proteins were aliquoted and frozen and stored at  $-80^{\circ}\text{C}$ . The protein was subsequently characterized by SDS gels, amino acid analysis for quantitation of protein, evaluated for endotoxin levels by an endotoxin assay, N-terminal protein sequencing to establish identity of protein, mass spectrometry to establish integrity of protein and other analytical procedures as necessary or desired.

#### Immunoglobulin Sequences and Constructs

Purified antibodies were subjected to amino acid sequencing using established methods. The N-terminal sequence was obtained and from that, primers were designed to clone out the Ig variable region (V) sequences by RT-PCR. The DNA fragment encoding the  $V_L$  or  $V_H$  region was cloned into a vector. Sequencing was performed using established methods, initially with primers to the vector. From the initial nucleotide sequence of the antibody that was obtained, primers specific to the antibody sequence were designed to obtain the remaining sequence.

The chimeric full length and Fab mouse-human anti-PSCA antibodies whose sequences are shown in Figure 13 were constructed by joining the murine  $V_L$  and  $V_H$  region domain sequences to the human C $\kappa$  and C $\gamma$ 1 sequences. The murine antibody V region fragments were cloned into vectors as restriction site cassettes which can be readily excised and ligated into preconstructed vectors containing human L and H constant regions of different isotypes to express chimeric mouse-human antibodies with PSCA specificity. The Fab chimeric chains were expressed from the vector called vegfchim derived from pUC119:Ig vector which is a pRK vector (described in EP 307,247).

Figure 12 shows the amino acid sequences of the light and heavy chain variable region domains ( $V_L$  and  $V_H$ ) of the antibodies from hybridoma clones 6F8.2F4, Asc# 2395 (SEQ ID NO. 3 & NO. 4); 5F2.4H4.1E3, Asc# 2403 (SEQ ID NO. 5 & NO. 6); 6B8.1D7.2B3, Asc#2761 (SEQ ID NO. 7 & NO. 8), and  $V_H$  of the antibody from clone 8D11.2E9 (Asc# 2399 (SEQ ID NO. 9). Figure 13 shows the amino acid sequences of the full length chimeric 2403 IgG including the signal peptide sequences (L chain is SEQ ID NO. 10; H chain is SEQ ID NO. 11) and the sequences of chimeric 6B8 Fab (L chain is SEQ ID NO. 12; H chain is SEQ ID NO. 13). Chimeric antibody 2403 was made by fusing the murine  $V_L$  and  $V_H$  sequences of Mab 5F2.4H4.1E3 (#2403) with human immunoglobulin C $\kappa$  and C $\gamma$ 1 sequences, respectively, in the mammalian cell expression vector, pRK. The construction of the pRK5 expression vector is disclosed in EP 307,247 published March 13, 1989. The pRK vectors encoding the chimeric 2403 H and L chains have been deposited with the ATCC as DNA pRK-2403H and DNA pRK-2403L (see X below). Mouse  $V_H$  was joined

to human C $\gamma$ 1 at sequential residue # Pro122 (denoted by / in sequence in FIG. 13). Mouse V<sub>L</sub> was joined to human C<sub>L</sub> at sequential residue # Arg113. The 2403 V<sub>H</sub> insert can be excised from pRK-2403H as an EcoRI-ApaI fragment or a ClaI-ApaI fragment of 430 bp. The 2403 V<sub>L</sub> was cloned into pRK-2403L as an EcoRI-CpoI fragment of 400 bp fragment. The pRK vectors encoding the chimeric 2716 H and L chains have been deposited with the ATCC as DNA pRK-2761H and DNA pRK-2761L (see X below). Likewise, the 2716 V<sub>L</sub> has been cloned into pRK-2761L as an EcoRI-CpoI fragment of 400 bp fragment. The 2761 V<sub>H</sub> insert can be excised from pRK-2761H as an EcoRI-ApaI fragment or a ClaI-ApaI fragment of 430 bp.

Chimeric 6B8 Fab L and H chains were made by fusing the murine V<sub>L</sub> and V<sub>H</sub> sequences of Mab 6B8.1D7.2B3 with human immunoglobulin C $\kappa$ , and C<sub>H</sub>1 domain of IgG1 sequences, respectively, in the vector called vegf4chim, and expressed in *E. coli*. The vegf4chim vector has an amp<sup>R</sup> selectable marker and the antibody sequences are operably linked to and expressed under, the alkaline phosphatase promoter.

These V region cassettes can be excised and ligated to constant region domains of the different human immunoglobulin isotypes to produce chimeras with different Fc properties. To produce anti-PSCA antibodies lacking effector functions such as ADCC, complement binding activity or Fc receptor binding, the murine V<sub>H</sub> regions are fused to the human C $\gamma$ 2 or C $\gamma$ 4 constant region sequences.

## II. Results

Table 2B summarizes the characteristics of the anti-PSCA antibodies as determined by the assays herein. In Table 2B, hu PSCA-*E. coli* refers to his-tagged PSCA expressed in *E. coli*; the protein was purified from the bacteria and then refolded as described above; + formalin indicates that formalin fixed cells were used as immunogen; prk5.hu PSCA refers to the naked cDNA carrying the hu PSCA gene in pRK5 plasmid. Xenomice (Abgenix, Fremont, California) are mice which have been genetically engineered to replace their endogenous murine Ig locus with the human germline immunoglobulin locus such that they make human antibodies. Human antibodies to PSCA are made by immunizing the Xenomice with *E. coli* expressed PSCA, intraperitoneally (i.p.) or via the footpad.

All anti-PSCA Mabs tested produced a significant shift in staining of the PSCA-transfected cells as assayed by FACS. Figure 1 (Mab 5F2) and Figure 2 (Mab 10C5) show representative staining results. Cells incubated with FITC-conjugated secondary antibody alone was used as negative control; these cells did not show any antibody binding activity. The anti-gD Mab 1766 gD, used as a positive control, showed significant binding to CHO cells expressing gD-huPSCA. Hybridoma supernatant containing the fully human Mab 10C5.6E4.6D1 also demonstrated strong binding to the cell surface. Murine anti-PSCA Mab 5F2 (#2403) was used as a positive control in this experiment and media as a negative control.

Murine Mab 6B8 (#2761) was tested for its ability to bind to various soluble forms of PSCA immobilized onto a plate and the results are shown in Figure 3. Mab 6B8 generated against denatured PSCA protein, demonstrated stronger affinity for the refolded huPSCA than for nonrefolded huPSCA. The Mab was also shown to bind to gD-huPSCA CHO soluble antigen, but with less affinity as compared to refolded and nonrefolded huPSCA. However, there was no binding to an irrelevant antigen TGF- $\beta$  and murine PSCA, suggesting that the antibody was specific to huPSCA.

From the cold competition binding assay (see Figure 4 and table immediately below), the binding affinity for the Mab 2403 was determined to be 12.9 nM.

5

$y = ((m1-m4)/(1+(m0/m3)^{m2}))+m4$		
	Value	Error
m1	40650	657.34
m2	1.0449	0.074328
m3	12.977	1.0636
m4	1865	1102.9
Chisq	1.9794e+06	NA
R	0.99921	NA

TABLE 2A

CLONE	MAbs ASC#	ANTIGEN	ADJUV.	DOSE	ROUTE	SCHEDULE	HOST
10E3.5A7.1A7	2359	hisPSCA - E. coli	Ribi	5 ug	FP X 8	2/week	Balb/c
6C3.4G12.1B7	2360	hisPSCA - E. coli	"	"	"	"	Balb/c
6F8.2F4	2395	hisPSCA - E. coli	Ribi	"	FP X 6	2/week	Balb/c
10A1.2F11	2396	hisPSCA - E. coli	"	"	"	"	Balb/c
2F2.2G7	2397	hisPSCA - E. coli	"	"	"	"	Balb/c
5B12.1C9	2398	hisPSCA - E. coli	"	"	"	"	Balb/c
8D11.2E9	2399	hisPSCA - E. coli	"	"	"	"	Balb/c
5A5.1D11	2400	hisPSCA - E. coli	"	"	"	"	Balb/c
2D2.1B12	2401	hisPSCA - E. coli	"	"	"	"	Balb/c
5F2.4H4.1E3	2403	hisPSCA - E. coli	"	"	"	"	Balb/c
7A6.1H4.2A5	2405	hisPSCA - E. coli	"	"	"	"	Balb/c
7B9.2C1.2H4.1D12	2429	hisPSCA - E. coli	"	"	"	"	Balb/c
12C5.1F10	2402	hisPSCA - E. coli	Ribi	"	FP X 7	2/week	Balb/c
2A4.3G2	2404	hisPSCA - E. coli	"	"	"	"	Balb/c
7B11.3H5.5C2	2430	hisPSCA - E. coli	"	"	"	"	Balb/c
N/A	N/A	hisPSCA - E. coli	Ribi	"	FP X 6	2/week	Balb/c
N/A	N/A	gDPSCA Ras 3T3	none	tumor	SQ	NA	Balb/c
N/A	N/A	gDPSCA - CHO membranes	Ribi	ND	FP X 6	1/week	Balb/c
5G1.1E5 (S/N)	N/A	hisPSCA - E. coli/formalin	Ribi	5 ug	FP X 10	2/week	Balb/c
N/A	N/A	gDPSCA - CHO	Ribi	5 ug	FP X 10	1/week	Xenomice
N/A	N/A	hisPSCA - E. coli	Ribi	5 ug	FP X 10	1/week	Xenomice
N/A	N/A	his Mu PSCA - E. coli	Ribi	10 ug	FP X 10	1/week	Lewis rats
N/A	N/A	hisPSCA - E. coli	Ribi	5 ug	FP X 5	1/week	Xenomice
N/A	N/A	hisPSCA - E. coli	Ribi	10 ug	IP X 5	1/week	Xenomice
4F9.1E8.1C2 (S/N)	2760	hisPSCA - E. coli/nonrefolded	Ribi	5 ug	FP X 5	1/week	Balb/c
6B8.1D7.2B3	2761	hisNonrefolded huPSCA - E. coli	Ribi	5 ug	FP X 5	1/week	Balb/c
3F9.1D4.2F1 (S/N)	2759	gDPSCA - CHO	Ribi	5 ug	FP X 10	2/week	Balb/c
2B12.1H6.1F9 (S/N)	2758	gDPSCA - CHO	Ribi	5 ug	FP X 11	2/week	Balb/c
N/A	N/A	his Mu PSCA - E. coli	Ribi	10 ug	FP X 5	1/week	Lewis rats
N/A	N/A	PSCA/GST - E. coli	Ribi	5 ug	FP X 8	1/week	Balb/c
NO TITERS	N/A	hPSCA-1/KLH (peptide)	Ribi	5 ug	FP X 7	1/week	Balb/C
NO TITERS	N/A	mPSCA-1/KLH (peptide)	Ribi	5 ug	FP X 7	1/week	Lewis rats
2 PARENTAL S/N	N/A	gDPSCA - HCT116	none	10 e6	FP X 10	1/week	Balb/c
N/A	N/A	gDPSCA - HCT116/formalin	none	10 e6	FP X 10	1/week	Balb/c
NO TITERS	N/A	prk5.hu.PSCA (cDNA)	none	100 ug	IM X 2	2/month	Balb/c



pending	pending	HCT116gDPSCA	none	10 e6	FP X 10	1/week	XenoMice
N/A	N/A	his Mu PSCA -E. coli	Ribi	10 ug	FP X 5	1/week	Lewis Rats
N/A	N/A	hisPSCA - E. coli/nonrefolded	Ribi	5 ug	FP X 5	1/week	XenoMice
N/A	N/A	PSCA/GST - E. coli	Ribi	5 ug	FP X 6	1/week	Balb/c
10C5.6E4.6D1 (S/N)	2910	hisPSCA-E.coli	Ribi	5 ug	IP X5	1/week	XenoMice
pending	pending	hisPSCA - E.coli/nonrefolded	Ribi	5 ug	FP X 5	1/week	XenoMice
pending	pending	his Mu PSCA - E. coli	Ribi	10 ug	FP X 5	1/week	Lewis Rats
pending	pending	PC3gDmPSCA Cells	none	10 X 106	FP X 10	1/week	Lewis Rats

TABLE 2B

CLONE	mAbs Asc#	ANTIGEN	SPECIES OR STRAIN	ISOTYPE	CAPTURE	FACS	IHC	EPITOPE	TUMOR TARGET	INTERNALIZE
10E3.5A7.1A7	2359	hu PSCA-E Coli	Balb/C	IgG1	Yes	+/-	+	E	ND	Yes
6C3.4G12.1B7	2360	hu PSCA-E Coli	Balb/C	IgG2a	Yes	+	-	B	ND	Yes
6F8.2F4	2395	hu PSCA-E Coli	Balb/C	IgG2b	Yes	++	-	A	Yes	Yes
10A1.2F11	2396	hu PSCA-E Coli	Balb/C	IgG2b	Yes	+	-	A	ND	ND
2F2.2G7	2397	hu PSCA-E Coli	Balb/C	IgG2b	No	-	+	ND	ND	ND
5B12.1C9	2398	hu PSCA-E Coli	Balb/C	IgG2b	Yes	-	+/-	C	ND	ND
8D11.2E9	2399	hu PSCA-E Coli	Balb/C	IgG2b	Yes	++	-	A	Yes	Yes
5A5.1D11	2400	hu PSCA-E Coli	Balb/C	IgG2a	Yes	+	-	ND	ND	ND
2D2.1B12	2401	hu PSCA-E Coli	Balb/C	IgG2a	Yes	-	-	D	ND	ND
5F2.4H4.1E3	2403	hu PSCA-E Coli	Balb/C	IgG2b	Yes	++	-	A	Yes	Yes
7A6.1H4.2A5	2405	hu PSCA-E Coli	Balb/C	IgG2a	Yes	+	-	A	ND	ND
7B9.2C1.2H4.1D12	2429	hu PSCA-E Coli	Balb/C	IgG2a	Yes	-	-	F	ND	ND
12C5.1F10	2402	hu PSCA-E Coli	Balb/C	IgG2b	Yes	+/-	-	A	ND	ND
2A4.3G2	2404	hu PSCA-E Coli	Balb/C	IgG2b	No	-	-	ND	ND	ND
7B11.3H5.5C2	2430	hu PSCA-E Coli	Balb/C	IgG1	Yes	+/-	+/-	ND	ND	ND
N/A	N/A	hu gDPSCA Ras 3T3	Balb/C							
N/A	N/A	hu PSCA-CHO membrane	Balb/C							
5G1.1E5	N/A	hu PSCA-E Coli + formalin	Balb/C	IgM	?	?	-			
N/A	N/A	hu gDPSCA - CHO	Xenomice	N/A	N/A	N/A	N/A			
N/A	N/A	hu PSCA-E Coli	Xenomice	N/A	N/A	N/A	N/A			
pending	N/A	muPSCA-E.coli	Lewis rats	IgG2a	Yes	-	-			
4F9.1F8.1C2 (S/N)	2760	hu PSCA-E Coli *	Balb/C	IgG2a	Yes	++	++		ND	
6B8.1D7.2B3	2761	hu PSCA-E Coli *	Balb/C	IgG2a	Yes	++	+++	B	Yes	Yes
3F9.1D4.2F1 (S/N)	2759	gDPSCA-CHO	Balb/C	IgG1	Yes	++	-	A	ND	
2B12.1H6.1F9 (S/N)	2758	gDPSCA-CHO	Balb/C	IgG1	Yes	++	+	A	ND	
2 parental S/N		gDPSCA-HCT116 cells	Balb/C	IgM		+				
pending		gDPSCA-HCT116 /formalin	Balb/C							
pending		gDPSCA-HCT116 cells	Xenomice							

no titers		prk5.hu PSCA (cDNA)	Balb/C	IgG2	Yes	++	+		
10C5.6E4.6D1(S/N)	2910	hu PSCA-E.coli	Xenomice						
N/A		hu PSCA-E.coli *	Xenomice						
pending		muPSCA-E.coli	Lewis rats						
pending		gDmuPSCA-PC3 cells	Lewis rats						

ND = not determined

Asc# = Ascites #

IHC = immunohistochemistry

hu PSCA = human PSCA

\*nonrefolded huPSCA

S/N = supernatant

IP = intraperitoneal

FP = via footpad

muPSCA = murine PSCA

### Example 2

This example demonstrated the binding and localization of anti-PSCA antibodies 2395 (6F8), 2399 (8D11), 2403 (5F2), 2761 (6B8) [ascites number followed by antibody no. in parenthesis; see Table 2B] to PSCA expressing tumor cells (MCF-7Her2gdPSCA and HCT-gdPSCA) *in vivo*. By labeling antibodies with the fluorescent dye, Cy3, antibody binding can be visualized by fluorescence microscopy. This technology was well established using Cy3 labeled Herceptin (anti-Her2 monoclonal antibody) in mice bearing Her2-expressing tumors. *In vivo* binding of the anti-PSCA antibodies were tested on tumor cells transfected with human gd-linked PSCA. Control antibodies included Cy3 labeled Herceptin as a positive control for the MCF-7Her2gdPSCA cells. Additionally, a Cy3 labeled anti-gd antibody, with very good binding activity, was used as a positive control for the PSCA antibodies. Transfected PSCA is gd linked, thus the level of gd protein directly correlates with PSCA protein expression. Cell lines not transfected with gdPSCA were used as negative controls.

## I. Materials and Methods

### Cell lines

HCT-116 (ATCC CCL-247) is a human colon tumor cell line; HCT-47 is a PSCA expressing clone derived from transfecting the parental HCT-116. MCF-7 (ATCC HTB-22; Soule, D. G. et al., J. Natl. Cancer Inst., 51:1409-1416, 1973) is a human breast tumor cell line which was previously transfected with and expresses Her2 (Benz et al. *Breast Cancer Research and Treatment* 24: 85-95 (1992)). PC3 (ATCC CRL-1435) is a human prostatic carcinoma cell line (Kaign et al. *Invest. Urol.*, 17: 16-23, 1979). These cells were transfected with human PSCA (huPSCA) or with gD-huPSCA. gD or gd refers to the Herpes Simplex virus glycoprotein D epitope tag. In the construction of gD-huPSCA, the signal sequence of PSCA was replaced with the sequence encoding the gD epitope tag; the gD-PSCA sequence was cloned into pRK vector. Her2 expression on the cell surface served as a control since Her2 antibody (HERCEPTIN®) is known to be able to localize to Her2-expressing tumor cells *in vivo*. From these transfections, human PSCA-expressing tumor cells were obtained.

### Antibody preparation

Refer to Table 2B for a list of the murine monoclonal anti-PSCA antibodies used in these experiments. The antibody names are derived from the first 3-4 characters of the clone producing the antibody. All antibodies were labeled using FluoroLink Cy3 monofunctional dye (Amersham Pharmacia, PA23001) according to the manufacturer's directions. Antibodies for the labeling reaction were in 0.1 M Sodium Carbonate pH 9.3 at a concentration of 1 mg/ml. One ml of the 1 mg/ml of antibody stock was added to the tube with Cy3 labeling mix, mixed thoroughly, and allowed to incubate at room temperature for 30 min on a shaker. Samples were wrapped in tin foil to reduce light exposure. NAP 10 columns (Amicon) were equilibrated with PBS and when the conjugation reaction was done, the reaction solution was added to the column. 1.5 ml PBS were added and the flow through was collected. Spectrophotometer readings were taken at A552 and A280. Protein concentration and dye to protein ratios were calculated as follows:

AB concentration:  $\{[A280 - (0.08 \times A552)] \times \text{dilution factor}\} / 1.4 = \text{mg/ml}$

Dye to Protein ratio:  $[1.13 \times (A552)] / [A280 - (0.08 \times A552)]$

After labeling, all antibodies were checked for binding by FACS analysis (see Table 2B).

### Tumor inoculations and growth

5 NCR nude female mice were implanted with Estrogen pellets and then inoculated subcutaneously with 20 million cells of MCF-7Her2gdPSCA or 20 million cells of non-transfected MCF-7 cell line as a negative control. Male NCR nude mice were inoculated subcutaneously with 5 million HCT-gdPSCA cells. The tumors are injected subcutaneously, so they are situated on the flank of the mouse. Using a caliper with a digital read out, tumor measurements were taken twice weekly until tumor volume reached 100 to 500 mm<sup>3</sup>.

### 10 Cy3 Antibody injection and tissue collection

Once tumors reached at least 150 mm<sup>3</sup> in volume, mice were injected IP with 10 mg/kg of one of the three Cy3 labeled anti-PSCA antibodies (6F2, 8D11, 5F2), a Cy3 anti-gd antibody, or Cy3 labeled HERCEPTIN. Twenty four hours later, mice were anesthetized and perfused with 1% paraformaldehyde. Tumor tissue and other organs were collected and frozen in OCT (Tissue-Tek O.C.T. Compound, Sakura  
15 Finetek U.S.A. Inc., Torrance CA 90504) cryoprotectant for antibody localization by fluorescence microscopy. Similar experiments were also carried out with Cy3 labeled 6B8 antibody.

### Fluorescence microscopy

20 Frozen samples were sectioned into 5 um sections and fluorescent mounting media with dapi (Vectastain) was applied. Dapi stains nuclei and provides a counterstain.

## II. Results

TABLE 3  
Antibody Preparations and FACS Analysis

ANTIBODY	dye/protein (D/P) ratio	Post-labeling FACS analysis
2395 (6F8)	4	shift
2399 (8D11)	4	shift
2403 (5F2)	3	shift
Herceptin (4D5)	5	shift
anti-gd (952)	5	shift

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To confirm that Cy3 labeling of the antibodies did not negatively affect their ability to bind antigen, the antibodies were tested for their binding specificity on cells by FACS analysis. In Table 3 "shift" refers to a shift in the cell population when screened for fluorescence and indicates that the Cy3 labeled antibodies maintain their abilities to bind their specific antigen.

TABLE 4  
Summary of Antibody Localization

ANTIBODY	MCF-7Her2gdPSCA Tumors staining positive	MCF-7 Tumors staining positive
2395 (6F8)	3 (n=4)	0 (n=3)
2399 (8D11)	2 (n=2)	0 (n=2)
2403 (5F2)	3 (n=4)	0 (n=3)
Herceptin(4D5)	2 (n=2)	0 (n=2)
anti-gd(952)	2 (n=2)	0 (n=2)
ANTIBODY	HCT-gdPSCA Tumors staining positive	
2395 (6F8)	1 (n=1)	
2399 (8D11)	2 (n=2)	

5

The results summarized in Table 4, demonstrated that the anti-PSCA antibodies were able to specifically localize and bind to PSCA-expressing tumor cells in vivo. No staining was detected in the lung or the kidney. Cy3 staining was observed in the liver for all PSCA antibodies, anti-gd, and Herceptin in what are most likely phagocytic Kupffer cells that have taken up the antibody. Non-malignant cells associated with the tumors, most likely macrophages, were also seen to stain positive for Cy3 for all antibodies on both the transfected and non-transfected cell lines. Additionally, antibody 6B8 was also shown to localize to PSCA-expressing PC-3 tumor cells but not on control-transfected PC3 tumor cells in vivo. The staining pattern was primarily membranous. Positive staining was also observed in macrophages in and adjacent to the tumor.

15

### Example 3

This example describes the ability of the anti-PSCA monoclonal antibodies of the invention, to be internalized by PSCA-expressing cells. These studies were performed using fluorescent or radiolabeled antibodies or antibodies with gold adducts. With the radiolabeled antibodies, quantitation of the amount of antibody internalized in each cell line was analyzed.

20

## I. Materials and Methods

### *In vitro* studies

Transfected cells expressing gD PSCA (HCT-47 and PC3-19 cells) and MCF7 cells expressing PSCA/HER2 (also referred to herein as MCF7/PSCA/HER2 cells) were grown on coverslips, incubated with a series of monoclonal anti-PSCA antibodies (10 µg/mL) for one hour (hr), washed in PBS, fixed in 3% PBS buffered formaldehyde, permeabilized with 1% Triton in PBS and labeled with Cy3-fluorescent labeled anti-mouse IgG. In control experiments, parental, untransfected cells were used. Immunolabeled cells were observed in a Molecular Dynamics confocal microscope.

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For electron microscopy, transfected NIH-3T3 expressing gD-PSCA were incubated at 4°C with 25 µg/ml of anti-gD antibody for one hr. In another set of experiments, CHO cells expressing PSCA were

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incubated at 4°C with 20µg/ml of 10E3 and 6C3 anti-PSCA antibodies. Following incubation with primary antibodies, the cells were treated with 10 nm gold adducts of goat anti-mouse IgG for one hour. The cells were switched to 37°C for 15 min and 1 hr before fixation in Karnovsky's fixative, and processed for electron microscopy. Thin sections were observed in a Philips CM12 equipped with a digitizing GATAN camera.

5 For electron microscopy autoradiography, 10A1 and 8D11 anti-PSCA antibodies were iodinated according to the manufacturer's instructions for Iodo-Gen. <sup>125</sup>I-10A1 and <sup>125</sup>I-8D11 anti-PSCA antibodies were incubated for 15 min, 1hr and 6hr with HCT116 gD PSCA cells (human colon tumor cell line transfected with gD human PSCA, described above). After incubation, the cells were washed and fixed in 2% formaldehyde, 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2 and postfixed in 1% osmium tetroxide. After dehydration and embedding in EPONATE 12 epoxy resin, thin sections were cut, coated with Ilford L4 emulsion. After exposure, the sections were developed in Microdol X, stained with uranyl acetate and lead citrate, and observed in a Philips CM12 electron microscope.

Internalization of 6B8 anti-PSCA antibody was studied by electron microscopy using PC3 cells expressing gD.huPSCA. 6B8 antibodies were iodinated according to manufacturer's instructions for Iodo-Gen. <sup>125</sup>I-6B8 antibodies were incubated for 15 min and 24 hr with PC3.gD.huPSCA clone4 cells. After incubation, the cells were washed and fixed with 2% formaldehyde, 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2 and postfixed with 1% osmium tetroxide. After dehydration and embedding in EPONATE 12 epoxy resin, thin sections were cut and coated with Ilford 1.4 emulsion. After exposure, the sections were developed in Microdol X, stained with uranyl acetate and lead citrate, and observed in a Philips CM 12 electron microscope.

A steady state internalization study was performed as follows: MCF7/Her2 cells (Fig. 11, right panel) are transfected with vector alone containing the puromycin selectable marker (PurR); this cell line served as negative control for internalization of the PSCA antibodies. MCF7/Her2/gD.PSCA cell line is described in Example 2. MCF7/Her2 and MCF7/Her2/gD.PSCA cells were incubated with one of the following antibodies for 5 hours at 37°C: 22nM <sup>125</sup>I labeled anti-PSCA monoclonal antibodies 2395, 2399, 2403, and 0.8nM <sup>125</sup>I labeled anti-Her2 antibody, Herceptin. Unbound antibody was removed and the cells were washed with cold medium. The amount of radioactivity bound to the cells was determined by incubating the cells in Acid Wash (2M Urea, 0.5M NaCl, 50mM Glycine pH2.4) for 10 minutes and then removing and counting the wash. The cells were then solubilized with Lysis Buffer (8M Urea, 3M glacial acetic acid) and the resulting lysates counted to determine the amount of radioactivity internalized. Using the specific activity of the radio-labeled antibodies and the number of cells in each assay sample, cpm were converted to molecule/cell.

### *In vivo studies*

35 MCF7/HER2/PSCA xenograft tumor bearing mice were dosed IP with Cy3 labeled 6F8, 5F2, or 8D11 PSCA antibodies. After 24 hrs, the tumors were removed and cryosectioned at 5, 10, 20 and 50µm and observed by confocal microscopy.

Anti-PSCA antibodies are labeled with <sup>125</sup>I as described for the *in vitro* experiment above. MCF-7/Her2/gdPSCA cells are prepared and inoculated into NCR nude mice and <sup>125</sup>I labeled anti-PSCA

antibodies, a  $^{125}\text{I}$ -anti-gd antibody, or  $^{125}\text{I}$  labeled HERCEPTIN® are injected into the mice as described in Example 2 above. Tumor cells are sectioned for analysis.

## II. Results

In the *in vitro* studies, confocal microscopy of immunolabeled HCT 47 gD PSCA, PC3-19 gD PSCA and MCF7/HER2/PSCA cells showed that these cells bind and internalize the following anti-PSCA monoclonal antibodies, 8D11, 10A1, 6C3, 6F8, 5F2, 7A6 and 10E3 to varying extents (See Figures 5-8). Internalization staining pattern was characterized by the presence of cytoplasmic vesicles and perinuclear organelles likely to correspond to the endosomes in proximity of the Golgi apparatus.

In the *in vivo* study, labeling can be seen on the cell surface of most of the cryosectioned cells and internalized into small vesicles near the cell surface in a small percentage of the cells.

EM internalization studies using the above-described 3T3 cells incubated with anti-gD antibodies visualized with 10 nm gold particles indicated that gD-PSCA was internalized and transported to the endosomal compartment. Gold particles were visualized in calveolae and multivesicular bodies. EM internalization studies using PSCA-transfected CHO cells also showed that the same pathway was involved in the internalization of both 10E3 and 6C3 antibodies (see Figure 9A-9D). The kinetics of PSCA internalization appeared to be rapid (15 min from the plasma membrane to the endosomes).

With  $^{125}\text{I}$ -10A1 and  $^{125}\text{I}$ -8D11 anti-PSCA antibodies, autoradiographic silver grains were observed associated with the cell membrane, particularly with the microvilli. Autoradiographic grains were also seen internalized within the cells as early as 15 minutes after addition of the  $^{125}\text{I}$ -labeled 10A1 and 8D11 antibodies. The internalization seen in the micrograph of Figure 10 is representative of the results of this experiment. Control HCT116 cells transfected with neo marker but not PSCA, showed no internalization.

The results with the 6B8 antibody were similar to those obtained with  $^{125}\text{I}$ -10A1 anti-PSCA antibody. Internalization of antibody 6B8 was observed by 15 minutes, continuing through the 24-hour time-point. Autoradiographic grains were first observed associated with the caveolae of the cell membrane and later internalized in the endosomes.

Figure 11 shows the steady state levels of internalization of three anti-PSCA antibodies [ascites no. 2395 (6F8), 2399 (8D11), 2403(5F2)] at 5 hours in Her2-expressing MCF7 cells transfected with gD-PSCA. Herceptin served as a control antibody. The number of molecules of anti-PSCA antibodies per cell bound versus free in the lysate, was quantitated.

## III. Conclusion

The antibodies directed against PSCA (6C3, 10E3, 10A1, 6F8, 5F2, 8D11, 6B8) were efficiently endocytosed and internalized both *in vivo* and *in vitro*. Ultrastructural analysis indicated that the internalization of 6C3, 10E3 and 6B8 likely takes place via calveolae. The antibodies accumulate in multivesicular bodies in close proximity to the Golgi apparatus. Thus, by different approaches, the anti-PSCA antibodies of the invention were shown to be internalized upon binding to PSCA on the cell surface.



#### Example 4

The abundance of PSCA in various normal and tumor tissues was assessed by determining from a database of cDNA libraries, the number of copies of the PSCA nucleotide sequence present in cDNA libraries representing genes expressed in different tissues and organs (see Figure 14). For each organ indicated in Figure 18 (x-axis), pools of several dozen cDNA libraries are represented. "All" means a mixture of the cDNA libraries representative of the different organs indicated in the figure, as well as cDNA libraries prepared from various tissues including bone, uterus, small intestine, thymus, stomach, connective tissue and unclassified tissue type. The relative abundance in normal and tumor organs is determined by the number of copies of the PSCA sequence in the pooled library divided by the total number of sequences in that pooled library and is represented in Figure 14 as #hits/100,000 random sequences. From the absolute abundance (Absabu), it is clear that PSCA expression is highly upregulated in prostate and bladder tumors.

#### Example 5

The anti-tumor activity of the anti-PSCA antibodies was assessed in vivo.

##### I. Materials and Methods

The human prostate cancer cell line, PC3, was obtained from the ATCC (ATCC CRL-1435). The cell line PC3.gDhuPSCA subclone was obtained by transfecting the parental PC3 line with pRK.gDPSCA.tkneo using the Fugene method (Roche Molecular Biochemical) and plating in 400ug/ml G418 (Life Technologies). After two weeks of growth, G418 resistant sub-clones were selected, expanded and assayed for gDPSCA expression. The cell line PC3.Neo was made by transfecting the parental PC3 line with the vector pRK.tkneo and selection in 400ug/ml G418. Stable colonies were pooled and expanded.

NCR nude mice (Taconic, Germantown, New York, USA) were injected subcutaneously with  $5 \times 10^6$  gDPSCA transfected PC-3 cells or neo transfected PC-3 cells. Anti-PSCA monoclonal antibodies 2395 (6F8), 2399 (8D11), 2403 (5F2), and 2761 (6B8) were administered intraperitoneally by injection at a dose of 10 mg/kg one day prior to cell inoculation. As a negative control, an anti-ragweed monoclonal antibody, MARG2 (Genentech), was administered by the same dosing regimen. Following cell inoculation, animals were treated biweekly with 10 mg/kg antibody. Tumor measurements were taken biweekly. The results of these experiments are shown in Figures 18-20.

In a separate experiment (posttreatment), tumors were allowed to establish to a certain size before the mice were treated with anti-PSCA antibody, mimicking the condition and treatment in prostate cancer patients. Optimally, the tumor size should be approximately  $75-100\text{mm}^3$  before antibody treatment. In this experiment, the NCR nude mice (Taconic, Germantown, New York, USA) were injected subcutaneously with  $5 \times 10^6$  gDPSCA transfected PC-3 cells and tumors were allowed to grow for 1 week. Mice were then grouped into four groups of nine mice, each group with a mean tumor volume of approximately  $200\text{mm}^3$ . The mice were then treated biweekly with 10 mg/kg anti-PSCA monoclonal antibodies 2395 (6F8), 2399 (8D11), 2761 (6B8), and 2403 (5F2) or anti-ragweed, MARG2 (Genentech). Tumor measurements were taken biweekly. The results of these experiments are shown in Figure 21.

## II. Results

The four anti-PSCA antibodies tested, i.e., 2395 (6F8), 2399 (8D11), 2403 (5F2), and 2761 (6B8), showed cytotoxic activity as naked (unconjugated) antibodies in killing the PSCA-expressing prostate cancer cells in vivo (see Figures 19-21), compared to the control ragweed antibody. The p values were significantly different from the control group at  $p < 0.05$  for 2395 and 2399 in Fig. 19, 2399, 2403 and 2761 in Fig. 20, and 2395 in Fig. 21. Tumoricidal activity was specific to PSCA expressing tumor cells (Figures 19-21) and absent in the control, PSCA-negative PC3 tumor cells (Figure 18). It was difficult to predict or control the growth rate of the PC3 tumor cells once injected into the mice, to timely initiate the antibody treatment. As a result, in these initial posttreatment experiments, the tumors were at a bigger ( $150\text{-}200\text{ mm}^3$ ) than optimum size ( $75\text{-}100\text{ mm}^3$ ) at the time of administration of the anti-PSCA antibody. Greater efficacy in tumor cytotoxicity can be expected if treatment with anti-PSCA antibody is initiated at a time when the tumor is smaller and of a more representative size and if the antibody dose was increased above 10mg/kg.

### Example 6

#### Cynomolgus Monkey PSCA

This example describes the cloning of the Cynomolgus monkey PSCA and shows that the present anti-human PSCA antibodies bind the highly homologous Cynomolgus PSCA and human PSCA with similar affinities. In addition to the high homology, we have found that Cynomolgus urothelium expresses PSCA in a manner that is identical to that observed for human urothelium, thus making this primate a good and appropriate animal model for anti-PSCA antibody toxicity studies. Naked and toxin-conjugated anti-PSCA antibodies will be tested in this model.

## I. Materials and Methods

### Cloning of Cynomolgus PSCA

RNA was purified from one cynomolgus monkey bladder. To clone PSCA, RT-PCR was performed using primers psca.2F (AAGGCTGTGCTGCTTGCCCT - SEQ ID NO: 14) and psca.1R (GAGTGGCACAAAGGCCTGGG - SEQ ID NO: 15). The resulting PCR products from two independent PCR reactions were subcloned into the PCR subcloning vector pCR2.1-TOPO (Invitrogen) and then sequenced.

#### **Affinity Measurements on Cell Surface PSCA**

The entire coding sequence for human and for Cynomolgus PSCA were cloned into an expression vector, pRK. Anti-PSCA monoclonal antibody 5F2 (ascites no. 2403) was labeled with  $^{125}\text{I}$  using the lactoperoxidase method. PC3 cells or CHO cells were transiently transfected with either pRK.gD.human psca or pRK.gD.cynomolgus psca using Eugene Reagent (Roche). After 2 days of transfection, the cells were replated in a 24 well dish and incubated in medium with various concentrations of unlabeled Mab 2403 and a constant amount of  $^{125}\text{I}$ -Mab 2403 for 16 hours at  $4^\circ\text{C}$ . Unbound antibody was removed and cells were washed with ice-cold medium. The amount of radioactivity bound was determined by solubilizing the cells

in 8M Urea/3M glacial acetic acid. The Kd value was calculated by scatchard analysis using the NewLigand program.

#### FACS Analysis:

5 COS Cells (African green monkey kidney cells) were transiently transfected with either vector pRK.human.pscA or pRK.cynomolgus.pscA. Two days after transfection, cells were removed from the culture dishes with 5mM EDTA and washed 1X with PBS. Cells were divided into several tubes and stained at 4 °C for one hour with 10ug/ml antibody 8D11 (ascites #2399) or 6B8. After washing 2X with PBS, cells were stained with FITC anti-mouse (ICN) antibodies and then analyzed using flow cytometry. Xenomouse antibodies (from 10C5 subclones 1D8, 3B8 and 6D1) were used at 100ul of hybridoma supernatant per reaction and were stained with FITC anti-human Kappa chain antibodies and again analyzed by flow cytometry. The results of the FACS analysis are shown in Table 5 below.

## II. Results

15 Seven subclones of the monkey PSCA were sequenced from which two types of sequences were obtained: type I (4 subclones) and type II (3 subclones). The DNA and translated amino acid sequences are shown in Figure 15 for type I (SEQ ID NO. 16 & 17) and Figure 16 for type II (SEQ ID NO. 18 & 19). As RNA was purified from an individual monkey bladder and the monkey was from the wild (not an inbred strain), these two types most likely represent different alleles of the same gene. Type I and Type II Cynomolgus PSCA show 98.64% identity at the DNA level, and 99.18% identity at the protein level with only a single amino acid difference the two, at amino acid 119 (Ser vs. Gly). PSCA is a GPI-anchored surface protein. In human PSCA, the cleavage position for GPI attachment is at amino acid 100. Assuming that cleavage and GPI-linkage occurs at a similar position in Cynomolgus PSCA, the mature type I and type II monkey PSCA would be 100% identical in amino acid sequence. Both types show approximately 94% identity to human PSCA across the entire coding sequence. Figure 17 compares the amino acid sequence of human (SEQ ID NO. 1) and Cynomolgus Type I PSCA (SEQ ID NO.17).

The mean FACS shifts (indicated in units of FITC) for anti-PSCA antibodies on human or Cynomolgus PSCA expressed in COS Cells are shown in Table 5 (for murine antibodies) and Table 6 (for Xenomouse antibodies). It was known from other studies that the 5F2, 8D11 and 6F8 antibodies have very similar binding affinities for human PSCA. Therefore, of these 3 antibodies, 5F2 was used as a representative in the cold competition assay. Table 7 compares the affinities of antibodies 5F2 and 6B8 for cell surface human and Cynomolgus PSCA as determined by cold competition assay. The results from PC3 and CHO transfected cells were similar. The results from FACS analysis and cold competition assay indicated that the murine and xenomouse human antibodies bound human and cynomolgus PSCA that was expressed in its native context on the cell surface, with equivalent affinity (within the margin of error).

TABLE 5 - Mean FACS Shifts

	<u>no Mab</u>	<u>8D11</u>	<u>6B8</u>
human	4.2	22.6	11
cynomolgus	3.9	21.9	10

5

TABLE 6 - Mean FACS Shifts

	<u>no Mab</u>	<u>1D8</u>	<u>3B8</u>	<u>6D1</u>
human	3.6	10	9.6	14.3
cynomolgus	3.8	10.9	12.3	14.4

TABLE 7 - Antibody Affinity for Cell Surface PSCA

10

<u>anti-psca</u> <u>mab</u>	<u>PSCA</u> <u>species</u>	<u>cell line</u>	<u>Kd</u>
5F2	human	PC3,qD,hu,psca clone 4	13.2
5F2	cynomolgus	PC3,cyn,psca clone 8	7
6B8	human	PC3,qD,hu,psca clone 4	21
6B8	cynomolgus	PC3,cyn,psca clone 8	3.3

**Example 7****Anti-PSCA Antibody - DM1 Conjugation**

15

This example describes the conjugation of an anti-PSCA antibody to the maytansinoid, DM1. DM1 is a prodrug that gets activated in the cell; it is a potent inhibitor of microtubule formation and disrupts mitosis. The structure of DM1 is shown in Figure 22. The structure of anti-PSCA antibody-DM1 conjugates is illustrated in Figure 23. For the following conjugation, the R group in Figure 22 is SH or a protected derivative thereof; particularly preferred is a disulfide derivative. Modifications may be made to this protocol, e.g., to optimize the pH or concentrations of reagents for individual antibodies; these modifications are routine in nature and will be familiar to a protein chemist of skill in the art.

20

**Modification of anti-PSCA antibody with SPP**

25

A purified anti-PSCA antibody (e.g., one of the anti-PSCA antibodies listed in Table 2B) is modified with N-succinimidyl-4-(2-pyridylthio) pentanoate (SPP) to introduce dithiopyridyl groups. The antibody (e.g., at about 6-10 mg/mL) in potassium phosphate buffer (PPB; e.g., 50 mM PPB at pH 6.5)

containing NaCl (50 mM) and EDTA (1 mM) is treated with SPP (e.g. at 5.3 molar equivalents in 2.3 mL ethanol). After incubation for 90 minutes under argon at ambient temperature, the reaction mixture is filtered following standard procedures e.g., by gel filtration through a Sephadex G25 column equilibrated with 35 mM sodium citrate, 154 mM NaCl, 2 mM EDTA. Antibody containing fractions are pooled and assayed. The degree of modification of the antibody is determined as described above.

#### Conjugation of anti-PSCA antibody-SPP-Py with DM1

The modified antibody (e.g., with 9.5  $\mu$ mol of releasable 2-thiopyridine groups) is diluted with the above 35 mM sodium citrate buffer, pH 6.5, to a final concentration of e.g., 2.5 mg/mL. DM1 (e.g., at 1.7 equivalents, 16.1  $\mu$ mol) in 3.0 mM dimethylacetamide (DMA, 3% v/v in the final reaction mixture) is then added to the antibody solution. The reaction proceeded at ambient temperature under argon for 20 hours.

The reaction is loaded on a gel filtration column, e.g., Sephacryl S300 gel filtration column (5.0 cm x 90.0 cm, 1.77 L) equilibrated with 35 mM sodium citrate, 154 mM NaCl, pH 6.5. The flow rate is at e.g., 5.0 mL/min and 65 fractions (20.0 mL each) are collected. The major peak comprises monomeric anti-PSCA antibody-DM1. Fractions around the major peak are pooled and assayed. The number of DM1 drug molecules linked per antibody molecule is determined, e.g., by measuring the absorbance at 252 nm and 280 nm, and found to be around 3-5 drug molecules per antibody molecule.

#### Example 8

##### Cytotoxicity of anti-PSCA-DM1 conjugates on cultured cells

Anti-PSCA antibodies #2761 (6B8) and #2399 (8D11) were conjugated to DM1 as described above. From the internalization studies, it was expected that the DM1 antibody conjugates should be efficiently taken up by PSCA expressing cells. Two anti-ragweed antibodies were used as controls for antigen specificity and immunoglobulin isotype. The results for the *in vitro* cytotoxicity of the first four DM1 conjugates are reported. Cytotoxicity was measured in clonogenic assays on PC3.Neo cells and PC3.gD human PSCA clone 4 cells. These two cell lines were shown to be equally sensitive to the non-conjugated DM1 (Fig. 24), suggesting that PC3.Neo cell line was a good antigen-negative control for the PC3.gD human PSCA clone 4 cells.

#### I. Method

Cells were plated in 6-well tissue culture grade plates in growth medium that contained or did not contain the antibody conjugate to be tested. Control cultures were plated at 500 cells/plate. Cultures that were exposed to the conjugate were plated at several densities between 500 and 5000 cells/well. The cells were then incubated at 37°C for 5 to 7 days to allow colony formation. The cells were fixed, stained with Crystal violet dye, and the colonies were scored. Plating efficiency (PE) was calculated as the number of colonies/number of cells plated. Surviving fraction was calculated as PE of treated cells/PE of non-treated cells. Plating efficiencies of control cultures were in the range of 40 to 60% for both the control and the PSCA-expressing cell line.

**II. Results:** (concentrations are given as those for the respective antibodies)

Conjugate	Reference #	Isotype	DM1/Ab	IC <sub>10</sub> , M
Anti-PSCA antibody #2761-DM1	1461-44	IgG <sub>2a</sub>	3.39	$\sim 1 \times 10^{-9}$
5 Anti-PSCA antibody #2399-DM1	1461-165	IgG <sub>2b</sub>	2.94	$\sim 3 \times 10^{-10}$
Anti-Ragweed antibody #1428-DM1	1461-154	IgG <sub>2a</sub>	2.68	not toxic
Anti-Ragweed antibody #1429-DM1	1461-175	IgG <sub>2b</sub>	1.85	not toxic

Antibody conjugate 2399-DM1 was cytotoxic for PC3.gDhuman PSCA clone 4 cells with an  
 10 IC<sub>10</sub>  $\sim 3 \times 10^{-10}$  M, continuous exposure (Fig. 25). An isotype-matched control, 1429-DM1 was not toxic for  
 the cells up to the highest concentration tested ( $3 \times 10^{-9}$  M). The second conjugate, 2761-DM1, was  
 cytotoxic for PC3.gDhuman PSCA clone 4 cells with an IC<sub>10</sub>  $\sim 1 \times 10^{-9}$  M, continuous exposure (Fig. 26).  
 An isotype-matched control, 1428-DM1 was not toxic for the cells up to the highest concentration tested ( $3 \times$   
 15  $10^{-9}$  M). None of the four antibody-DM1 conjugates were toxic for PC3.Neo cells up to the highest  
 concentration tested ( $3 \times 10^{-9}$  M), see Fig. 27.

**Conclusion**

Antibody conjugates 2399-DM1 and 2761-DM1 were specifically cytotoxic towards the PSCA  
 antigen-positive cells.

**Example 9**Effect of Antibody-DM1 Conjugates on Tumor Growth in Vivo**I. Materials and Methods**

Female NCR nude mice (Taconic, Inc.) 6-8 weeks of age were injected with  $5 \times 10^6$  PC-3gdPSCA  
 cells subcutaneously in a volume of 0.2 ml on Day 1. Tumors were allowed to grow for 5 days and then were  
 25 measured in two dimensions using a caliper. Tumor volume was expressed in mm<sup>3</sup> using the formula:  $V =$   
 $0.5a \times b^2$ , where a and b are the long and the short diameters of the tumor, respectively. Mice were grouped  
 into 6 groups of 7 mice with a mean tumor volume of 160 mm<sup>3</sup>. On Day 6, antibody treatments were started.  
 Naked (non-DM1 conjugated) anti-PSCA antibodies 2399 (8D11), 2761 (6B8) and the anti-Ragweed  
 negative control antibody, 1428, were injected intraperitoneally (IP) twice a week at a dose of 10 mg/kg. This  
 30 treatment regimen was continued through out the experiment. The same antibodies conjugated to the  
 maytansinoid cytotoxin, DM1, were injected intravenously (IV) at a concentration of 75 ug/kg of DM1.  
 DM1 antibodies were administered twice a week for a total of 8 doses. Tumors were measured twice a week  
 through out the experiment. Table 8 below shows the dosing regimen.

TABLE 8

Group	Antibody	DM1 dose	AB dose	Schedule
1	2399-naked IP	N/A	10 mg/kg	2X week entire duration
2	2761-naked IP	N/A	10 mg/kg	2X week entire duration
3	Anti-Ragweed Naked (1428)	N/A	10 mg/kg	2X week

	IP			entire duration
4	2399-DM1 IV	75 ug/kg	5.5 mg/kg	2X week 8 total doses
5	2761-DM1 IV	75 ug/kg	5 mg/kg	2X week 8 total doses
6	Anti-ragweed-DM1(1428) IV	75 ug/kg	6.1 mg/kg	2 X week 8 total doses

## Results

As shown in Figure 28, by day 26 of treatment, the human prostate tumors (PSCA-expressing cells) were virtually eliminated in the animals treated with DM1-conjugated anti-PSCA antibodies (2399 and 2761) compared to both the non-conjugated anti-PSCA antibodies and the DM1-antiragweed control antibody. Within each group of 7 animals treated with DM1-2399 or DM1-2761, four of the animals had no detectable tumor. For the three animals with remaining tumor, the average tumor size was just 6 mm<sup>3</sup> compared to a tumor volume of about 550 mm<sup>3</sup> for the DM1-ragweed control antibody group. DM1 treatments will continue for one more dose ending on day 29. On day 33, mice from all groups are sacrificed and tumor tissues are collected for histological analysis. Other tissues including liver, kidney and bladder are collected and examined for evidence of cytotoxicity related to antibody treatments.

## Example 10

### PSCA Expression Analysis Using Tissue Microarray

The expression of human PSCA in normal tissues and malignant tumors was investigated using isotopic in-situ hybridization (ISH) on multi-tissue arrays. A tissue microarray (TMA) is a paraffin block which typically contains between one hundred to more than one thousand individual tissue samples. TMAs are constructed by taking small biopsy samples from "donor" tissues embedded in paraffin blocks and re-embedding the biopsies together in a single "recipient" block to form an array.

#### I. Method

PCR primers (upper- 5' ACC CAC GCG TCC GGC TGC TT 3' [SEQ ID NO. 24] and lower- 5' CGG GGG ACA CCA CGG ACC AGA 3' [SEQ ID NO. 25]) were designed to amplify a 768 bp fragment of human PSCA. Primers included extensions encoding 27-nucleotide T7 or T3 RNA polymerase initiation sites to allow in vitro transcription of sense or antisense probes, respectively, from the amplified products. Tumor microarray slides were deparaffinized, deproteinized in 20 µg/ml of proteinase K for 15 minutes at 37 °C, and further processed for *in situ* hybridization. <sup>33</sup>P-UTP labeled sense and antisense probes were hybridized to the sections at 55°C overnight. Unbound probe was removed by incubation in 20mg/ml RNase A for 30 min at 37°C, followed by a high stringency wash at 55°C in 0.1 X SSC for 2 hours and dehydration through graded concentrations of ethanol. The slides were dipped in NBT2 nuclear track emulsion (Eastman Kodak), exposed in sealed plastic slide boxes containing desiccant for 4 weeks at 4°C, developed and counterstained with hematoxylin and eosin.

#### II. Results

The results of the ISH assay for normal prostate and prostate tumors are as summarized in Table 9.

TABLE 9

Tissue	In-situ Hybridization		
	Negative	Weakly Positive	Strongly Positive
Normal Prostate (n=29)	55%	28%	17%
Normal Urothelium (n=4)	0%	25%	75%
Primary Prostate Cancer (n=97)	48%	27%	25%
Metastatic Prostate Cancer (n=28)	22%	39%	39%

The group of primary prostate cancer contains seven cases of prostatic intraepithelial neoplasia (PIN; one case is negative, one case is weakly positive and five cases are strongly positive for PSCA). The group of metastatic prostate cancers consists of 25 cases of lymph node metastases, two cases of liver metastases (both cases are strongly positive for PSCA) and one case of a brain metastasis (weakly positive for PSCA). Preliminary results on an additional prostate multi-tissue array (normal prostate, primary and metastatic prostate cancer) confirm these numbers. The results for other tumor types (lung and colon) are preliminary and appear to indicate that approximately 10% of lung tumors and 5% of colon tumors express PSCA at a weak or moderate level.

#### Example 11

##### PSCA distribution in tumors and normal tissue

Example 11 describes the distribution of PSCA in tumors and normal tissue as evaluated by immunohistochemistry (IHC) using the anti-PSCA mAbs herein. A series of primary, organ-confined prostate cancers is evaluated for expression of PSCA. The expression of PSCA is also assayed in frozen sections of breast, lung, colon and kidney tumors, and in colon carcinoma cell lines. Separately, Taqman<sup>TM</sup> analysis for PSCA is being performed on some of the same cases used for this IHC study. TaqMan<sup>TM</sup> is a fluorescent PCR-based technique which makes use of the 5' exonuclease activity of Taq DNA polymerase enzyme to monitor gene amplification in real time.

##### **Staining Procedure for PSCA on Frozen Sections**

Five micron thick frozen sections are cut from fresh frozen tumor tissue on a Leica cryostat and stored at -20°C until further use.

Sections are dried thoroughly and fixed in Acetone/Ethanol for 5 minutes. They are then rinsed twice with phosphate-buffered saline (PBS), for 5 minutes each. Endogenous peroxidase activity is quenched using the glucose oxidase/glucose method at 37°C for 60 minutes. This is followed by rinses with PBS, 2 changes for 5 minutes each. Blocking for endogenous biotin is done using Vector Avidin/Biotin Blocking Kit. Blocking for endogenous immunoglobulin binding sites is done with 10% normal horse serum for 30 minutes. Blocking serum is not washed off.

The sections are incubated with anti-PSCA murine antibody at 10ug/ml diluted in blocking serum for 60 minutes at room temperature (RT). A mouse isotype control antibody (Zymed) is used for negative control. The sections are rinsed with PBS twice for 5 minutes each. The detection of specifically bound primary antibody is accomplished with biotinylated horse anti-mouse Ig. The sections are incubated with diluted biotinylated horse anti-mouse IgG 1:200 in blocking serum for 30 minutes. The sections are rinsed with PBS twice for 5 minutes each, incubated with diluted ABC reagent for 30 minutes and again rinsed with PBS twice for 5 minutes each. Sections are incubated with Pierce Metal Enhanced DAB for 5 minutes, then



rinsed with tap water for 5 minutes, counterstained with Mayer's hematoxylin for 1 minute, rinsed with water, transferred into 70%, 95% and 100% Ethanol, followed by xylene and coverslipped. The sections are then dehydrated, cleared and mounted in synthetic mounting media.

## 5 Results

PSCA expression in malignant epithelial cells is seen. The level of expression ranges from weak to very strong, the proportion of malignant cells positive ranges from less than 10% to >95%. PSCA mRNA has previously been demonstrated in a case of colorectal adenocarcinoma by in-situ hybridization.

## 10 Example 12

Animal models are useful for understanding pathogenetic mechanisms and for the development of novel therapeutics. This study reports the pattern of expression of the murine PSCA in fetal and adult tissues by *in situ* hybridization, as well as a murine model for human prostate adenocarcinoma.

15 Structurally, PSCA is a GPI-linked membrane glycoprotein with homology to members of the Thy-1/Ly-6 family of proteins. It is most closely related to Sca2, a cell surface maker for immature lymphocytes (Noda S. et al. Journal of Experimental Medicine 1996; 183:2355-60). Expression of human PSCA RNA in normal tissues was originally described not only as tissue specific, but essentially limited to prostatic basal cells. The authors inferred from this latter observation and from the structural homology to Sca2 that PSCA may represent a "stem" cell antigen.

20 The murine homologue has been shown to demonstrate 70% identity to human PSCA at the nucleotide and the amino acid level. (Reiter RE et al., 1998, Proc. Nat. Acad. Sci. 95:1735-40). One well established animal model for human prostate cancer is the Transgenic Adenocarcinoma of the Mouse Prostate™ (TRAMP™) (Greenberg NM, 1995, PNAS 92:3439-43). Though there are significant anatomical differences in the mouse prostate compared to that of the human, there are inherent common characteristics including secretory function and hormonal regulation. In the TRAMP™ model, the minimal promoter of the rat probasin gene targets expression of the SV40 large T- antigen to the prostatic epithelium. All male TRAMP mice progress to prostate cancer usually by 8-12 weeks (Gingrich JR et al., 1997, Cancer Research 57:4687-91). The autochthonous TRAMP™ model is particularly relevant to human prostate carcinoma in that the development of the cancer is specific to the prostatic epithelium and is initially regulated by androgens. Furthermore, metastases to distant sites eventually occur (Gingrich JR et al., 1996, Cancer Research 56:4096-102; Gingrich JR et al., 1999, Prostate Cancer & Prostatic Diseases 2:70-75).

## I. Materials and methods

### Mice and Tissue Collection

35 Male and female adult CD-1 mice between the ages of 10 and 24 weeks were obtained from Charles River laboratories. Pregnant mice were ordered for the collection of embryos. The day of isolation with copulatory plug was considered day 1 of embryonic development. Fetal tissues examined by ISH included: E10, E13, E14, E15, E17 and E18. Adult tissues examined included: tissues of the male urogenital system, female urinary bladder and kidney, pubescent mammary gland, 14 day pregnant mammary gland, lactating mammary gland, liver, heart, skin and intestine. All tissues were fixed in 4% formalin and paraffin-

embedded. Breeder pairs of TRAMP<sup>TM</sup> transgenic mice were obtained from Dr. Norman Greenberg (Baylor College of Medicine, Houston, TX). Urogenital tissues from wild type litter mate C57BL/6 were taken at age 12 and 24 for comparison to age matched TRAMP<sup>TM</sup> transgenic mice. By the age of 12 weeks, TRAMP<sup>TM</sup> mice have typically progressed to PIN and or well differentiated tumors. Nine TRAMP<sup>TM</sup> male mice between the ages of 12 to 39 weeks of age were sacrificed and tissues were collected for routine histology and ISH studies. The histological grade of prostate cancer was determined in according to previously published studies in the TRAMP<sup>TM</sup> model (Gingrich JR et al., 1999, Prostate Cancer & Prostatic Diseases 2:70-75).

#### Cloning of the Murine PSCA Orthologue

Full-length cDNA for murine PSCA was amplified from mouse 17-day embryo Marathon-Ready cDNA (Clontech). Primers were designed based on EST sequences present in Genbank. Sense primer (5' ACT ATG AAG CTT TGC AGC TCA TCC CTT CAC AAT CG 3' (SEQ ID NO. 20)) and anti-sense primer in the 3' untranslated region (5' GAA TTC GGA TCC ACC ATG AAG ACC GTC TTC TTT CTC CTG CTG 3' (SEQ ID NO. 21)) were used resulting in a 420 bp fragment that was subsequently cloned into the PCR subcloning vector PCR2.1TOPO (Invitrogen). Clones were confirmed by DNA sequencing.

#### In Situ Hybridization

PCR primers (upper- 5' CCT GCT GGC CAC CTA CT 3' and lower- 5' CCT TCA CAA TCG GGC TAT 3' - SEQ ID NOS. 22 & 23, respectively) were designed to amplify a 388 bp fragment of murine PSCA. Primers included extensions encoding 27-nucleotide T7 or T3 RNA polymerase initiation sites to allow in vitro transcription of sense or antisense probes, respectively, from the amplified products (Lu et al. Cell Vision 1994, 1:169-176). Five µm thick sections were deparaffinized, deproteinized in 4 µg/ml of proteinase K for 30 minutes at 37 °C, and further processed for *in situ* hybridization. <sup>33</sup>P-UTP labeled sense and antisense probes were hybridized to the sections at 55°C overnight. Unbound probe was removed by incubation in 20 mg/ml RNase A for 30 minutes at 37 °C, followed by a high stringency wash at 55 °C in 0.1 X SSC for 2 hours and dehydration through graded concentrations of ethanol. The slides were dipped in NBT2 nuclear track emulsion (Eastman Kodak), exposed in sealed plastic slide boxes containing desiccant for 4 weeks at 4°C, developed and counterstained with hematoxylin and eosin. The *in situ* hybridization was routinely performed on duplicate sections with sense and antisense probes. No significant hybridization signal was observed on sections hybridized with the sense probe.

#### Results

In summary of the results, murine PSCA is expressed during fetal development in the urogenital sinus, skin and gastrointestinal tract. The expression in these tissues is restricted to the most superficial cell layer. In the adult mouse, expression is highest in the mucosal lining of the urinary tract. In the normal adult prostate, expression of PSCA is detected exclusively in the secretory epithelium. Examination of PSCA during carcinogenesis of the murine prostate in the TRAMP<sup>TM</sup> model showed a markedly increased expression in areas of neoplasia.

These studies demonstrate that expression of murine PSCA is neither prostate specific or consistent with it being a stem cell marker. The results clearly indicate that PSCA expression in epithelial surfaces is

restricted to the adluminal or superficial cell layer and not, as reported for the human gene, to basal cells. Cells of the adluminal outer layer typically display a high degree of differentiation, but unlike stem cells, low proliferative or self-renewal capability. The designation of this molecule as a "stem cell antigen" is therefore questionable. These results suggest that the pattern of expression of murine PSCA in normal tissues and in tissues of murine prostatic adenocarcinoma is different from published data on human prostate adenocarcinomas. Most likely, the differences in PSCA expression between the TRAMP<sup>TM</sup> tumors and human prostatic adenocarcinoma either indicate species-specific regulation of PSCA expression or are related to the type of carcinogenic event at play.

The strongest expression of PSCA is detected in the urothelium of the developing and mature urinary tract. In the adult organism the expression appears continuous from the renal pelvis throughout the urethra. Prostatic expression is patchy, but increases noticeably at the juncture of the large prostatic ducts and urethra. It is tempting to speculate, whether this finding is related to urinary reflux into these structures providing a potential clue to the function of this protein. A common feature of the epithelial surfaces exhibiting PSCA expression is the fact that they line structures which are in continuous contact with fluids, mucous or secretions. This is obviously the case for urothelium, however, this notion also holds true for the observation that significant PSCA expression is present in the amniotic membrane, anal canal, oropharynx, esophagus and skin of the developing fetus. Not to be limited by any one hypothesis or mechanism, it is likely that additional denominators are at play, because other fluid-exposed epithelial tissues such as the fetal tracheobronchial tree, do not express PSCA. Whether the expression in fetal skin is temporally regulated is difficult to determine at this point as the number of embryos evaluated is small. However it appears that expression of PSCA in skin is no longer required in postnatal, extrauterine life. The pattern of expression within multilayered epithelial surfaces suggests that PSCA may function as a barrier or buffer against external insults. Further studies, such as the generation of PSCA knockout mice would be helpful to clarify the primary function of PSCA in normal tissue.

The rather selective expression of PSCA in fetal and adult tissues makes the mouse a suitable model to determine the physiologic function of this protein. Though PSCA is lost in the advanced tumor stages, the TRAMP<sup>TM</sup> model may still represent a potentially useful tool to study PSCA as a target for prostate cancer.

## CONCLUSION

The working examples above demonstrated that the anti-PSCA antibodies of the invention effectively targeted PSCA-expressing cancer cells *in vivo* and that the GPI-linked PSCA molecule and the antibody bound to it were rapidly internalized upon the binding of the antibody to a PSCA-expressing cancer cell. The anti-PSCA antibodies showed specificity and efficacy in tumor cell killing and arresting tumor growth *in vivo*. Cytotoxicity of the tumor cells was greatly enhanced using toxin-conjugated antibody, specifically the maytansinoid, DM1, which is a toxin that acts intracellularly. Treatment with DM1-conjugated anti-PSCA antibody completely eliminated the PSCA-expressing, human prostate tumor cells *in vivo* (see Example 9).

## IX. References

References cited within this application, including patents, published applications and other publications, are hereby incorporated by reference.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology and the like, which are within the skill of the art. Such techniques are explained fully in the literature. See *e.g.*, Molecular Cloning: A Laboratory Manual, (J. Sambrook *et al.*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989); Current Protocols in Molecular Biology (F. Ausubel *et al.*, eds., 1987 updated); Essential Molecular Biology (T. Brown ed., IRL Press 1991); Gene Expression Technology (Goeddel ed., Academic Press 1991); Methods for Cloning and Analysis of Eukaryotic Genes (A. Bothwell *et al.* eds., Bartlett Publ. 1990); Gene Transfer and Expression (M. Kriegler, Stockton Press 1990); Recombinant DNA Methodology II (R. Wu *et al.* eds., Academic Press 1995); PCR: A Practical Approach (M. McPherson *et al.*, IRL Press at Oxford University Press 1991); Oligonucleotide Synthesis (M. Gait ed., 1984); Cell Culture for Biochemists (R. Adams ed., Elsevier Science Publishers 1990); Gene Transfer Vectors for Mammalian Cells (J. Miller & M. Calos eds., 1987); Mammalian Cell Biotechnology (M. Butler ed., 1991); Animal Cell Culture (J. Pollard *et al.* eds., Humana Press 1990); Culture of Animal Cells, 2<sup>nd</sup> Ed. (R. Freshney *et al.* eds., Alan R. Liss 1987); Flow Cytometry and Sorting (M. Melamed *et al.* eds., Wiley-Liss 1990); the series Methods in Enzymology (Academic Press, Inc.); Wirth M. and Hauser H. (1993); Immunochemistry in Practice, 3rd edition, A. Johnstone & R. Thorpe, Blackwell Science, Cambridge, MA, 1996; Techniques in Immunocytochemistry, (G. Bullock & P. Petrusz eds., Academic Press 1982, 1983, 1985, 1989); Handbook of Experimental Immunology, (D. Weir & C. Blackwell, eds.); Current Protocols in Immunology (J. Coligan *et al.* eds. 1991); Immunoassay (E. P. Diamandis & T.K. Christopoulos, eds., Academic Press, Inc., 1996); Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; Ed Harlow and David Lane, Antibodies A laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988; Antibody Engineering, 2<sup>nd</sup> edition (C. Borrebaeck, ed., Oxford University Press, 1995); and the series Annual Review of Immunology; the series Advances in Immunology.

## 25 X. Deposit of Cell Lines and DNA

The following hybridoma cell lines were deposited with the American Type Culture Collection (ATCC), located at 10801 University Boulevard, Manassas, Virginia 20110-2209, U.S.A., and accorded the accession numbers:

	<u>Hybridoma</u>	<u>ATCC Accession No.</u>	<u>Deposit Date</u>
30	PSCA.10E3	PTA-717	September 16, 1999
	PSCA.6F8	PTA-718	September 16, 1999
	PSCA.8D11	PTA-719	September 16, 1999
	PSCA.5F2	PTA-720	September 16, 1999
	PSCA.6C3	PTA-880	October 26, 1999
35	PSCA.6B8	PTA-2265	July 25, 2000
	PSCA.10C5	PTA-2264	July 25, 2000
The following vector DNAs (see Example 1) were deposited with the ATCC			
	pRK-2403L		October __, 2000
	pRK-2716L		October __, 2000
40	pRK-2403H		October __, 2000
	pRK-2716H		October __, 2000

The names of the deposited hybridoma cell lines above have been shortened for convenience of reference; these hybridomas correspond to the clones (with their full names) listed in Table 2B.

5 This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

15 The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strains are not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws. The making of these deposits is by no means an admission that deposits are required to enable the invention.

20

WHAT IS CLAIMED IS:

1. An isolated anti-prostate stem cell antigen (PSCA) antibody that internalizes upon binding to PSCA on a mammalian cell in vivo.
- 5 2. The antibody of claim 1 which is a monoclonal antibody.
3. The antibody of claim 1 which is an antibody fragment.
4. The antibody of claim 1 which is a chimeric or a humanized antibody.
5. The antibody of claim 2 which is produced by a hybridoma selected from the group of hybridomas deposited under American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, or PTA-2264, or which has the amino acid sequence of SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, or SEQ ID NO.13.
- 10 6. The antibody of claim 2, wherein the antibody competes for binding to the same epitope as the epitope bound by the monoclonal antibody produced by a hybridoma selected from the group of hybridomas deposited under the American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2265, PTA-2264.
7. The antibody of claim 2 which is conjugated to a growth inhibitory agent.
8. The antibody of claim 2 which is conjugated to a cytotoxic agent.
9. The antibody of claim 8 wherein the cytotoxic agent is selected from the group consisting of toxins, antibiotics, radioactive isotopes and nucleolytic enzymes.
- 20 10. The antibody of claim 9 wherein the cytotoxic agent is a toxin.
11. The antibody of claim 10, wherein the toxin is selected from the group consisting of maytansinoid or calicheamicin
12. The antibody of claim 11, wherein the toxin is a maytansinoid.
13. The antibody of claim 12, wherein the maytansinoid has the structure shown in FIG. 22.
- 25 14. The antibody of claim 2, wherein the mammalian cell is a cancer cell.
15. An anti-PSCA monoclonal antibody that inhibits the growth of PSCA-expressing cancer cells in vivo.
16. The antibody of claim 15, wherein the antibody internalizes upon binding to PSCA on the cancer cell.
- 30 17. The antibody of claim 16 which is a humanized or human antibody.
18. The antibody of claim 17 which is produced in bacteria.
19. The antibody of claim 15, which is a humanized form of an anti-PSCA antibody produced by a hybridoma selected from the group of hybridomas having ATCC accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, or PTA-2265.
- 35 20. The antibody of claim 15, wherein the cancer cells are from a cancer selected from the group consisting of prostate cancer, bladder cancer and lung cancer.
21. The antibody of claim 20, wherein the cancer is prostate cancer.
22. An isolated nucleic acid comprising a sequence that encodes a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO.3, SEQ ID NO.4, SEQ ID NO.5, SEQ ID NO.6, SEQ ID NO.7, SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, or SEQ ID NO.13.
- 40

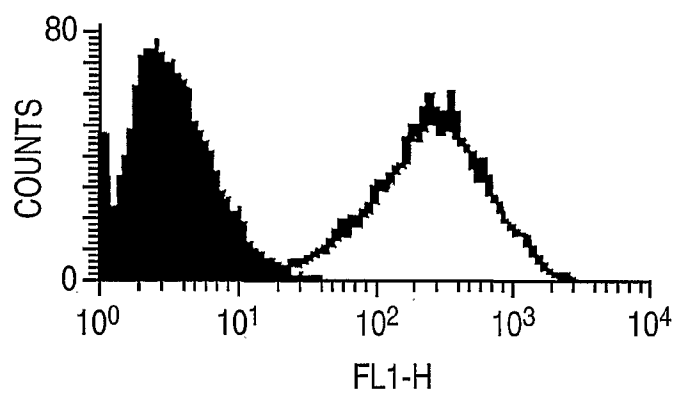
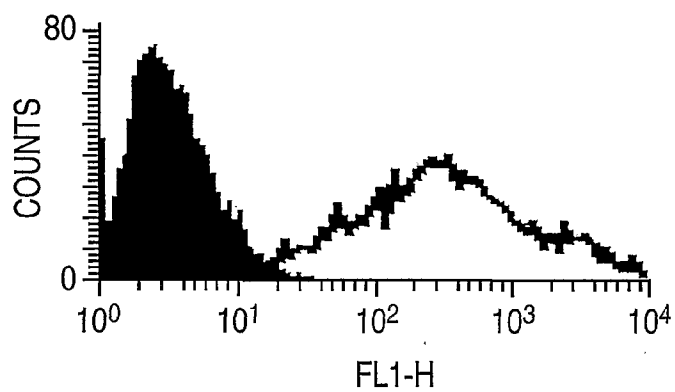
23. An expression vector comprising the nucleic acid of claim 22 operably linked to an expression regulatory sequence.
24. A cell that produces the antibody of claim 2.
25. The cell of claim 24, wherein the cell is selected from the group of hybridoma cells deposited under American Type Culture Collection accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, PTA-2264, or PTA-2265.
26. A host cell comprising the vector of claim 23.
27. The host cell of claim 26 which is a bacterial cell.
28. A method of producing the antibody of claim 2 comprising culturing the cell of claim 24 and recovering the antibody from the cell culture.
29. A composition comprising the antibody of claim 2 or claim 15, and a carrier.
30. The composition of claim 29, wherein the antibody is conjugated to a cytotoxic agent.
31. The composition of claim 30, wherein the cytotoxic agent is a maytansinoid.
32. The composition of claim 31, wherein the antibody is a human or humanized antibody and the carrier is a pharmaceutical carrier.
33. The composition of claim 32, wherein the humanized antibody is a humanized form of an anti-PSCA antibody produced by a hybridoma selected from the group of hybridomas having ATCC accession number PTA-717, PTA-718, PTA-719, PTA-720, PTA-880, or PTA-2265.
34. A method of killing a PSCA-expressing cancer cell, comprising contacting the cancer cell with the antibody of claim 1, thereby killing the cancer cell.
35. The method of claim 34, wherein the cancer cell is selected from the group consisting of prostate cancer, bladder cancer and lung cancer cell.
36. The method of claim 35, wherein the cancer cell is a prostate cancer cell.
37. The method of claim 36, wherein the prostate cancer is androgen independent.
38. The method of claim 36, wherein the cancer cell is from metastatic prostate cancer.
39. The method of claim 34, wherein the antibody is an antibody fragment.
40. The method of claim 34, wherein the antibody is a humanized antibody.
41. The method of claim 40, wherein the antibody is conjugated to a cytotoxic agent.
42. The method of claim 41, wherein the cytotoxic agent is a toxin selected from the group consisting of maytansinoid or calicheamicin.
43. The method of claim 42, wherein the cytotoxic agent is a maytansinoid.
44. The method of claim 43, wherein the antibody is a humanized form of the antibody produced by a hybridoma selected from the group of hybridomas having ATCC accession number PTA-718, PTA-719, PTA-720, PTA-880, or PTA-2265.
45. The method of claim 41, wherein the cytotoxic agent is a radioactive isotope.
46. A method of alleviating a PSCA-expressing cancer in a mammal, comprising administering a therapeutically effective amount of the antibody of claim 15 to the mammal.
47. The method of claim 46, wherein the cancer is selected from the group consisting of prostate cancer, bladder cancer and lung cancer.
48. The method of claim 47, wherein the cancer is a prostate cancer.

49. The method of claim 48 wherein the prostate cancer is androgen independent or metastatic prostate cancer.
50. The method of claim 46, wherein the antibody is a humanized antibody.
51. The method of claim 50, wherein the antibody is conjugated to a cytotoxic agent.
- 5 52. The method of claim 51, wherein the cytotoxic agent is a maytansinoid.
53. The method of claim 52, wherein the maytansinoid has the structure shown in FIG. 22.
54. The method of claim 46, wherein the antibody is administered in conjunction with at least one chemotherapeutic agent.
55. The method of claim 54, wherein the chemotherapeutic agent is paclitaxel or derivatives thereof.
- 10 56. An article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody of claim 2, and further comprising a package insert indicating that the composition can be used to treat prostate cancer.

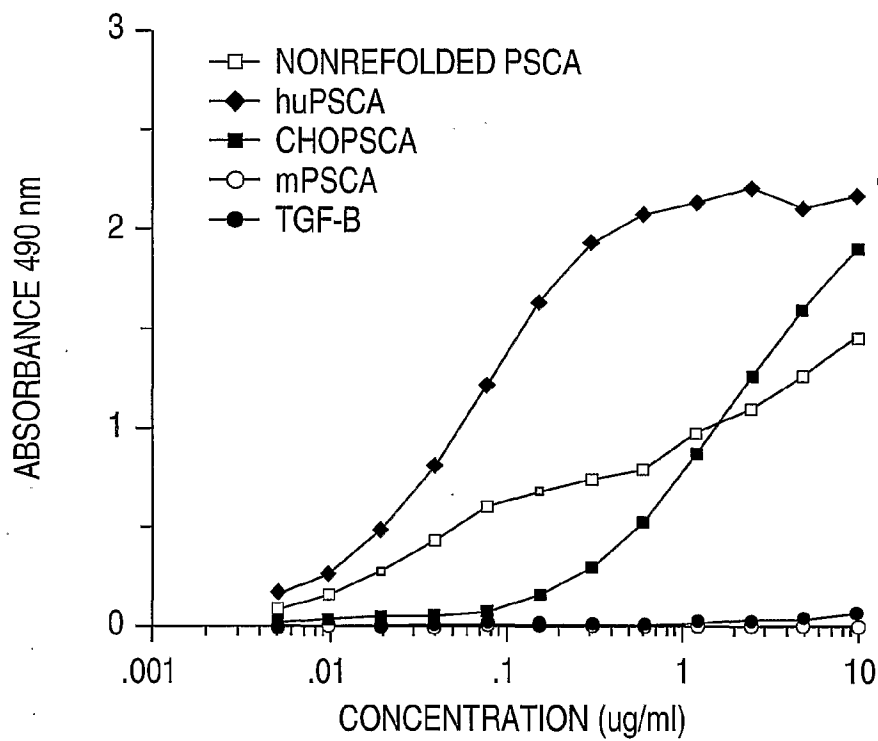
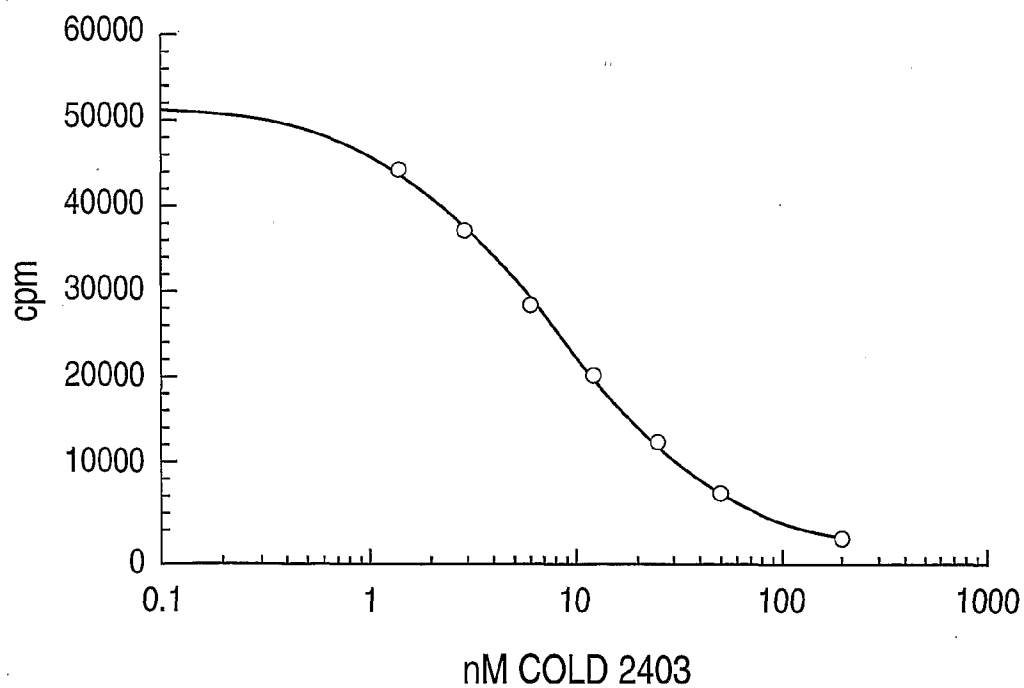
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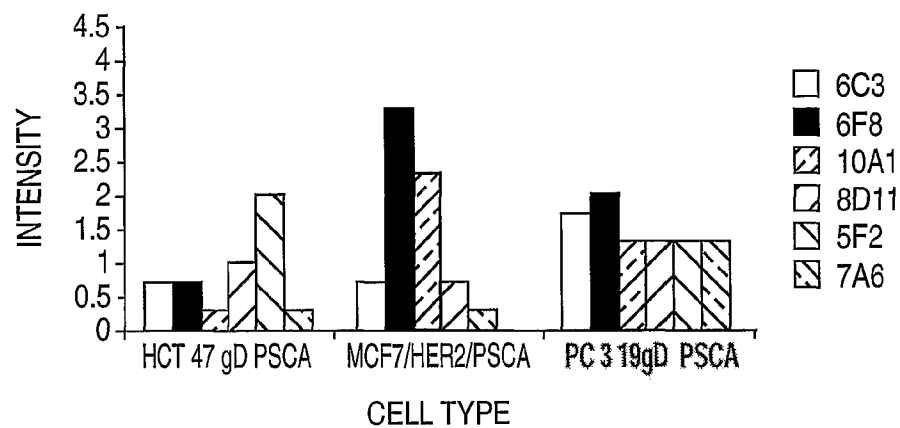
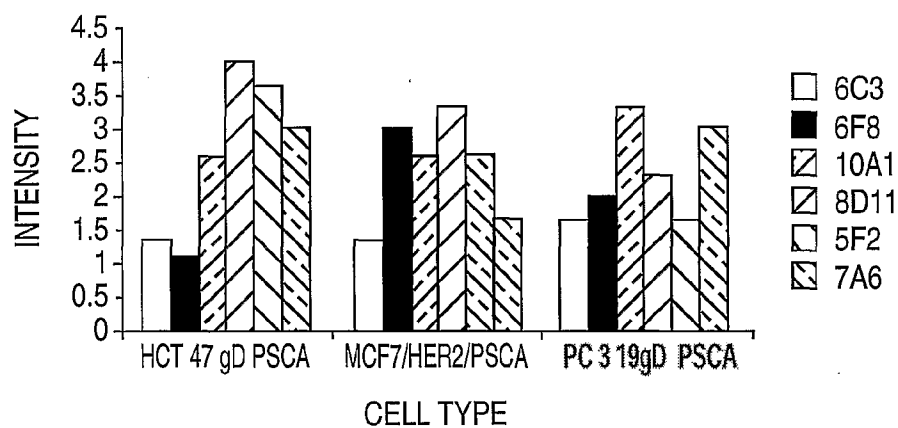
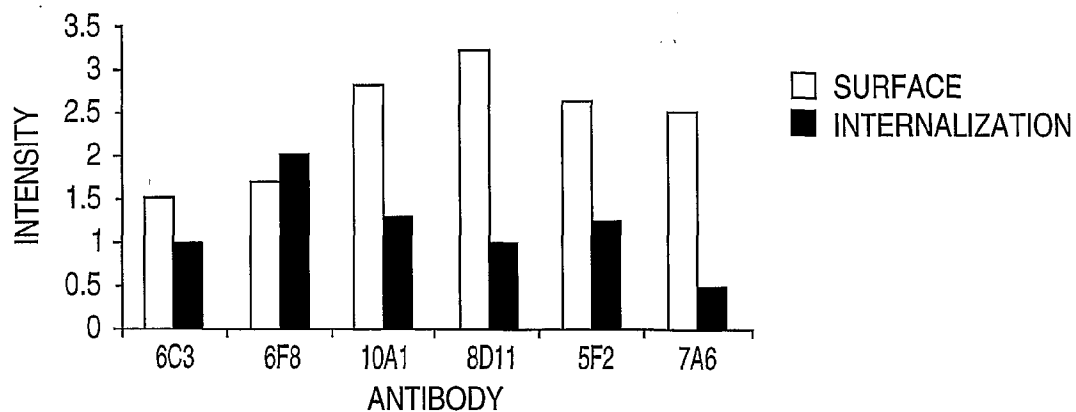
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**FIG. 1****FIG. 2**

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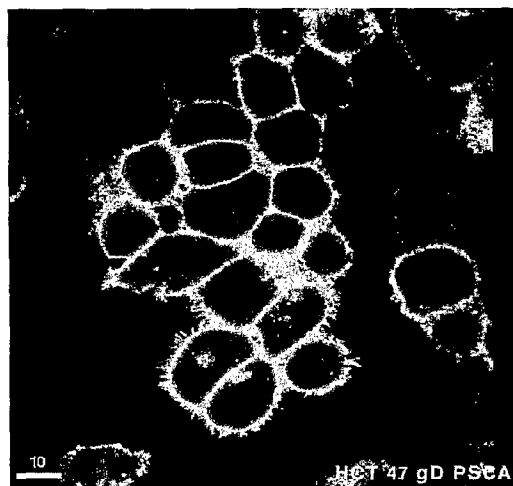
**FIG. 3****FIG. 4**

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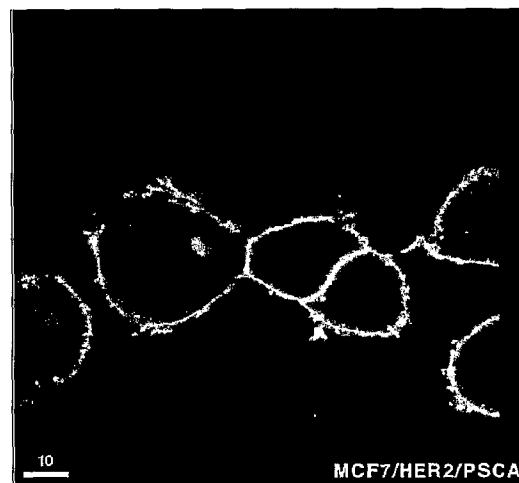
**FIG. 5****FIG. 6****FIG. 7**

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**FIG. 8A**



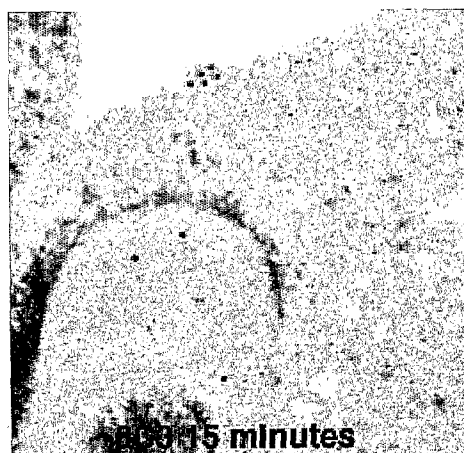
**FIG. 8B**



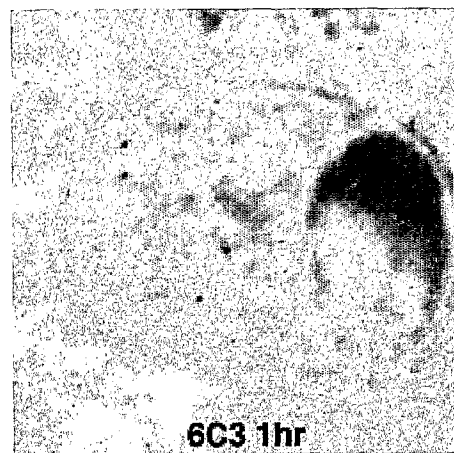
**FIG. 8C**



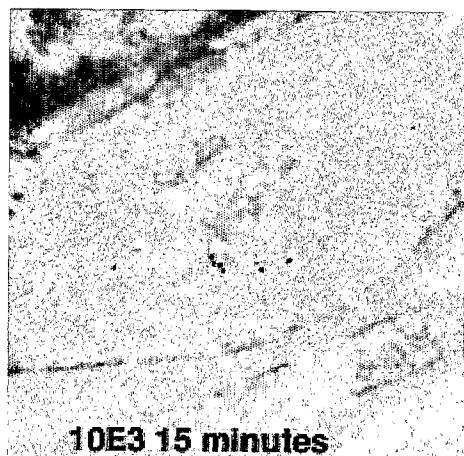
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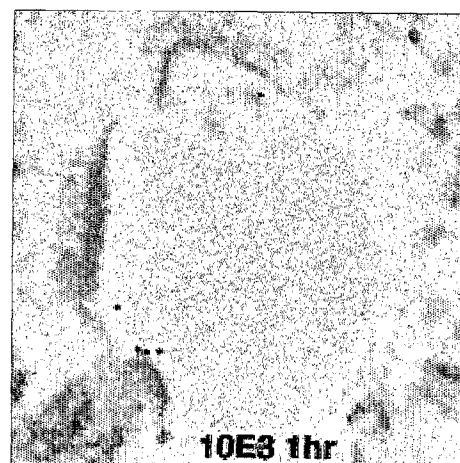
**FIG. 9A**



**FIG. 9B**

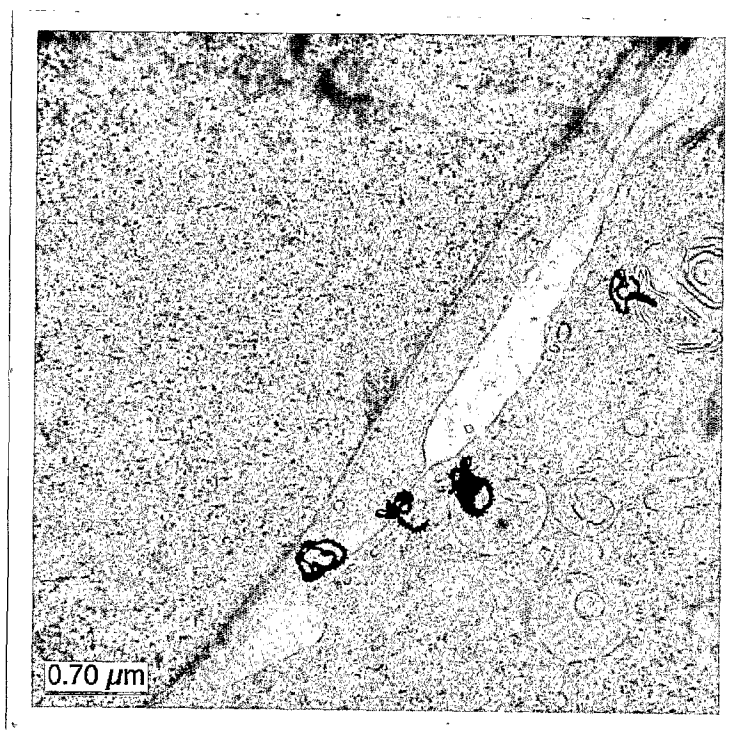


**FIG. 9C**



**FIG. 9D**

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**FIG. 10**

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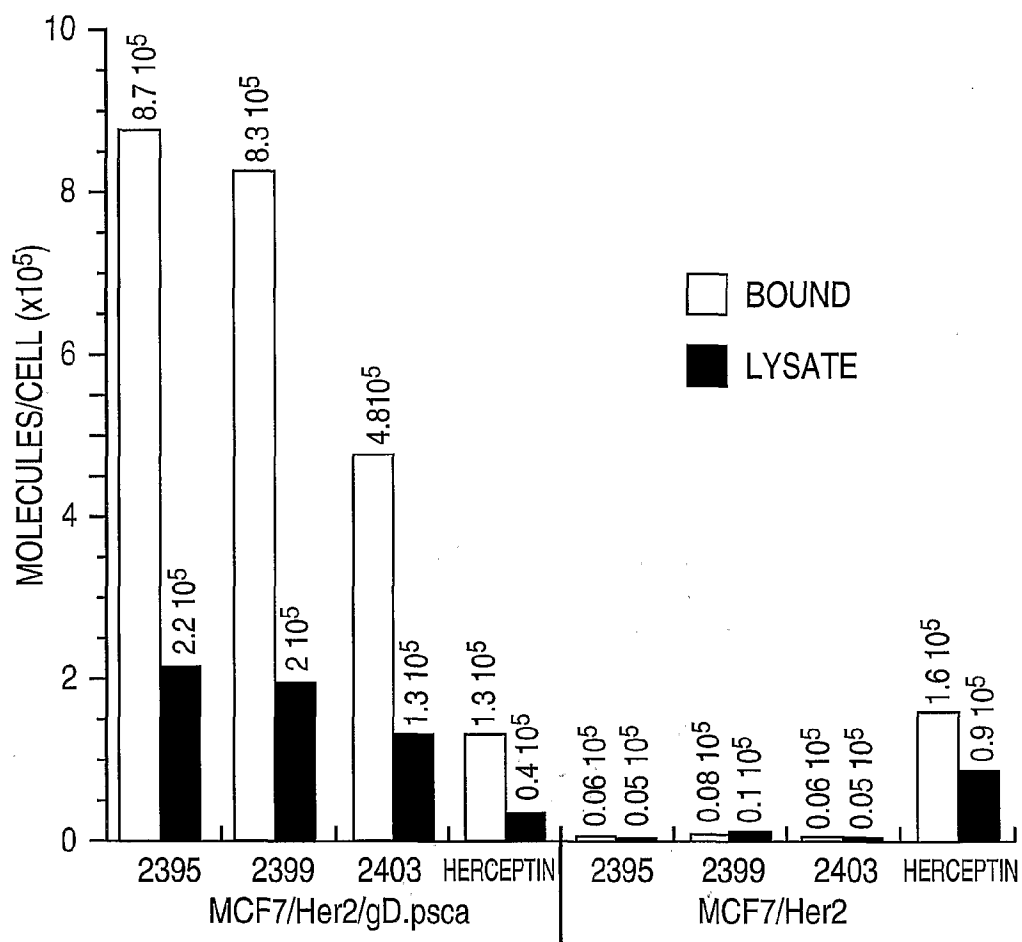


FIG. 11

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HEAVY CHAIN SEQUENCES OF ANTI-PSCA MABsCDR1CDR2

2395 : N-----ELVKPGAPVKLSCKASGYTFTNYWMNWKQRPGRGLEWIGRIDPSXXXXXXQTFKDKA  
 2399 : N-----PGAELVKPGAPVKLSCKASGYTFTNYWMNWKQRPGRGRGLEWIGRIDPSDSETQYNQTFKDKA  
 2403 : N-QVQVQQPGAELVKPGAPVKLSCKASGYTFTNYWLNWKQRPGRGRGLEWIGRIDPSDSEIHYDQKFKDKA  
 2761 : N-EVQLQQSGPDLEKPGASVKISCKPSGNSFTGYIYIHWVKQSHGKSLEWIGRVDPPNNGFTSYNQKFKGKA

CDR3

2395 : TLTVDKSSSTAYIQLSSLTSEDSAVYYCAITAAIAMDYWGQTSVTVSSAKTTGPS-C  
 2399 : TLTVDKSSSTAYIQLSSLTSEDSAVYYCAITAAIAMDYWGQTSVTVSSAKTTGPS-C  
 2403 : TLTVDKSSSTAYIQLSSLTSEDSAVYYCALTGIYAMAYWGQTSVTVSSAKTTGPS-C  
 2761 : ILTVDKSSSTAYMELRSLTSEDSAVYYCVG-NFFDS--WGQGTTLTVSSAKTTGPS-C

LIGHT CHAIN SEQUENCES OF ANTI-PSCA MABsCDR1CDR2

2395 : N-----SVSISCRSSKSLHSHNGNTYLYWFLQRPQGSPQLLIYRMSNLA SGVPDRFS  
 2403 : N-DIVMTQAAPSPVPTPGESVSISCRSSKSLHSHNGNTYLYWFLQRPQGSPQLLIYRMSNLA SGVPDRFS  
 2761 : N-DVVMTQTPTLTLSVTIGQPASISCKSSQSLDSDGKTYLNLWLLQRPQGSPKRLIYLVSTLDSGVDPDRFT

CDR3

2395 : GSGSGTVFTLRLSRVEAEDGVYYCMQHLESPFTFGSGGTKLEIKR-C  
 2403 : GSGSGTAFTLRLSRVEAEDGVYYCLQHLEYPYTFGGGTKLEIKR-C  
 2761 : GSGSGTDFTLKLSRVEAEDLGYYCWQGTHTFFRTFGGGGTKLEIKR-C

FIG. 12



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**chimeric 2403 (5F2.4H4.1E3) Light Chain**

signal peptide MGWSCILFLVATATGVHS

DIVMTQAAPSPVTPGESVSISCRSSKSLLSHNGNTYLYWFLQRPQGSPQLLYRMSNLASGVDPDRFSGSGGTAFTLRISRVEAEDVG  
 VYYCLQHLEYPYTFGGGKLELK/RTVAAPSVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS  
 TYSLSSITLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

**chimeric 2403 (5F2.4H4.1E3) IgG Heavy Chain**

signal peptide MGWSCILFLVATATGVHS

QVQQQPGAEVLKPGAPVKLSCKASGYTFTNYWLNWVKQRPGRGLEWIGRIDPSDSEIHVDQKFKDKATLTVDKSSSTAYIQLSSLT  
 SEDSAVYYCALTGIYAMAYWGQTSVTSSAKTTG/PSVHPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL  
 QSSGLYSLSSVTVPSSSLGTQTYICNVNHHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV  
 DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYIT  
 LPDSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYT  
 QKSLSLSPGK

**chimeric 2761 (6B8.1D7.2B3) Fab - Light Chain**

DVVMTQTPLTSLVTIGQPASISCKSSQSLLDSDGKTYLNLWLLQRPQGSPKRLIYLVSTLDSGVDPDRFTSGSGGTDFTLKISRVEAEDLGV  
 YYCWQGTTHFPRTFGGKLEIKR [V<sub>L</sub>/C<sub>k</sub> junction]

TVAAPSVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTYLSSTLTLISKADYEKHKVYACET  
 HQGLSSPVTKSFNRGEC

**chimeric 2761 (6B8.1D7.2B3) Fab - Heavy chain**

EVQLQQSGPDLEKPGASVKISCKPSGNSFTGYYYHWVKQSHGKSLEWIGRVDPNNGFTSYNQKFKGKAILTVDKSSSTAYMELRSLTSE  
 DSAVYYCVGNFFDSWGQGTTLTVSSA [V<sub>H</sub>/C<sub>γ</sub>1 junction]  
 KTTGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHHKPSN  
 TKVDKKVEPKSCDKTHT

**FIG. 13**

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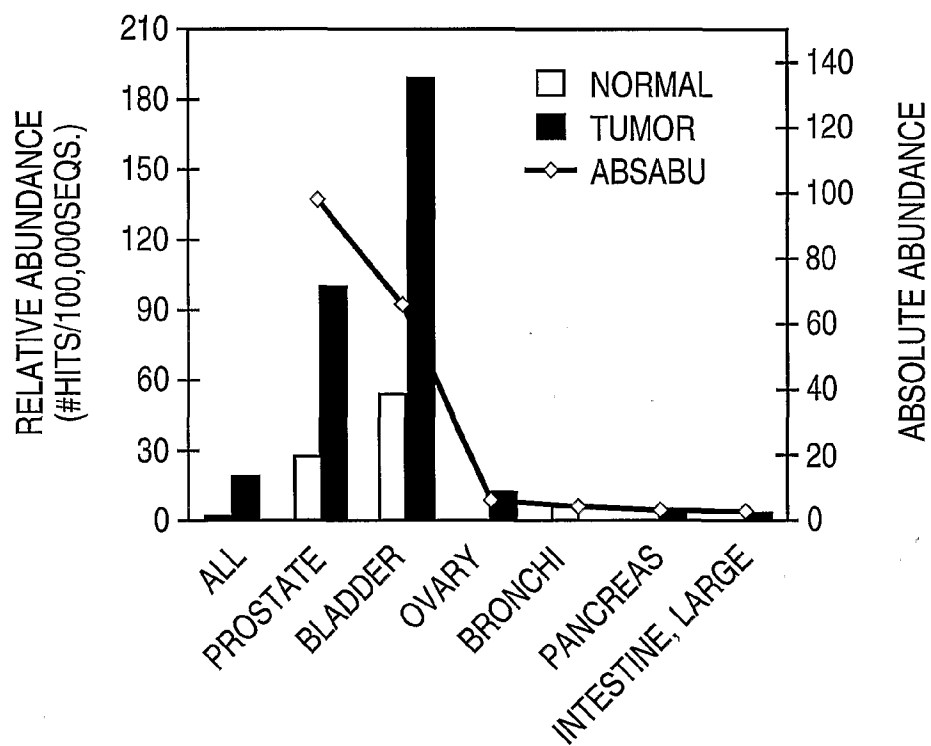


FIG. 14

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1 ATGAAGGCTG TGCTGCTTGC CCTGTTGATG GCAGGCTTGG CCCTGCAGCC AGGCACTGCC  
TACTTCCGAC ACGACGAACG GGACAACCTAC CGTCCGAACC GGGACGTCGG TCCGTGACGG  
1 M K A V L L A L L M A G L A L Q P G T A

61 CTGCTGTGCT ACTCCTGCAA GGCCCAGGTG AGCAACGAGG ACTGCCTGAA TGTGGAGAAC  
GACGACACGA TGAGGACGTT CCGGGTCCAC TCGTTGCTCC TGACGGACTT ACACCTCTTG  
21 L L C Y S C K A Q V S N E D C L N V E N

121 TGCACGCAGC CGGAGGAGCA GTGCTGGACC GAGCGCATCC GCGCCGTGGG CCTCCTGACC  
ACGTGCGTCG GCCTCCTCGT CACGACCTGG CTCGCGTAGG CGCGGCACCC GGAGGACTGG  
41 C T Q P E E Q C W T E R I R A V G L L T

181 GTCATCAGCA AAGGCTGCAG CTCAAACCTGC GTGGATGACT CACAGGACTA CTACGTGGGC  
CAGTAGTCGT TTCCGACGTC GAGTTTGACG CACCTACTGA GTGTCCTGAT GATGCACCCG  
61 V I S K G C S S N C V D D S Q D Y Y V G

241 AAGAAGAACA TCACCTGCTG TGACACCGAC TTGTGCAACG CCAGCGGGGC CCATGCACTG  
TTCTTCTTGT AGTGGACGAC ACTGTGGCTG AACACGTTGC GGTGCGCCCG GGTACGTGAC  
81 K K N I T C C D T D L C N A S G A H A L

301 CAGCCGGGCTG CTGCCATCCT GGCACCTGCTC CCTGCACTCA GTCTGCTGCT TTGGAGCCCC  
GTCGGCCGAC GACGGTAGGA CCGTGACGAG GGACGTGAGT CAGACGACGA AACCTCGGGG  
101 Q P A A A I L A L L P A L S L L L W S P

361 AGACAGCTGT AG  
TCTGTCGACA TC  
121 R Q L O

FIG. 15

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1  ATGAAGGCTG TGCTGCTTGC CCTGTTGATG GCAGGCTTGG CCCTGCAGCC AGGCACTGCC
   TACTTCCGAC ACGACGAACG GGACAACCTAC CGTCCGAACC GGGACGTCGG TCCGTGACGG
1  M K A V L L A L L M A G L A L Q P G T A

61 CTGTTGTGCT ACTCCTGCAA GGCCCAGGTG AGCAACGAGG ACTGCCTGAA TGTGGAGAAC
   GACAACACGA TGAGGACGTT CCGGGTCCAC TCGTTGCTCC TGACGGACTT ACACCTCTTG
21 L L C Y S C K A Q V S N E D C L N V E N

121 TGCACGCAGC CGGAGGAGCA GTGCTGGACC GAGCGCATCC GCGCCGTGGG CCTCCTGACC
   ACGTGCGTCG GCCTCCTCGT CACGACCTGG CTCGCGTAGG CGCGGCACCC GGAGGACTGG
41 C T Q P E E Q C W T E R I R A V G L L T

181 GTCATCAGCA AAGGCTGCAG CTCAAACTGC GTGGATGACT CACAGGACTA CTACGTGGGC
   CAGTAGTCGT TTCCGACGTC GAGTTTGACG CACCTACTGA GTGTCCTGAT GATGCACCCG
61 V I S K G C S S N C V D D S Q D Y Y V G

241 AAGAAGAACA TCACCTGCTG TGACACCGAC TTGTGCAACG CCAGCGGGGC CCATGCCCTG
   TTCTTCTTGT AGTGGACGAC ACTGTGGCTG AACACGTTGC GGTGCGCCCG GGTACGGGAC
81 K K N I T C C D T D L C N A S G A H A L

301 CAGCCAGCTG CTGCCATCCT GGCACGCTC CCTGCACTCA GCCTGCTGCT TTGGGGCCCC
   GTCGGTCGAC GACGGTAGGA CCGTGACGAG GGACGTGAGT CGGACGACGA AACCCCGGGG
101 Q P A A A I L A L L P A L S L L L W G P

361 AGACAGCTGT AG
   TCTGTGACA TC
121 R Q L O

```

FIG. 16

	10	20	30	40	50
HUMAN	MKAVLLALLMAGLALQPGTALLCYSCKAQVSNEDECLQVENCTQLGEQCWT				
	*****				
CYNOMOLGUS	MKAVLLALLMAGLALQPGTALLCYSCKAQVSNEDECLNVENCTQPEEQCW				
	10	20	30	40	50

	60	70	80	90	100
HUMAN	ARIRAVGLLTVISKGCSLNCVDDSDQYYVGKKNITCCDTDLNAGAHAL				
	*****				
CYNOMOLGUS	ERIRAVGLLTVISKGCSLNCVDDSDQYYVGKKNITCCDTDLNAGAHAL				
	60	70	80	90	100

	110	120
HUMAN	QPAAAILALLPALGLLWGPQL	
	*****	
CYNOMOLGUS	QPAAAILALLPALSLLLWSPRQL	
	110	120

FIG. 17

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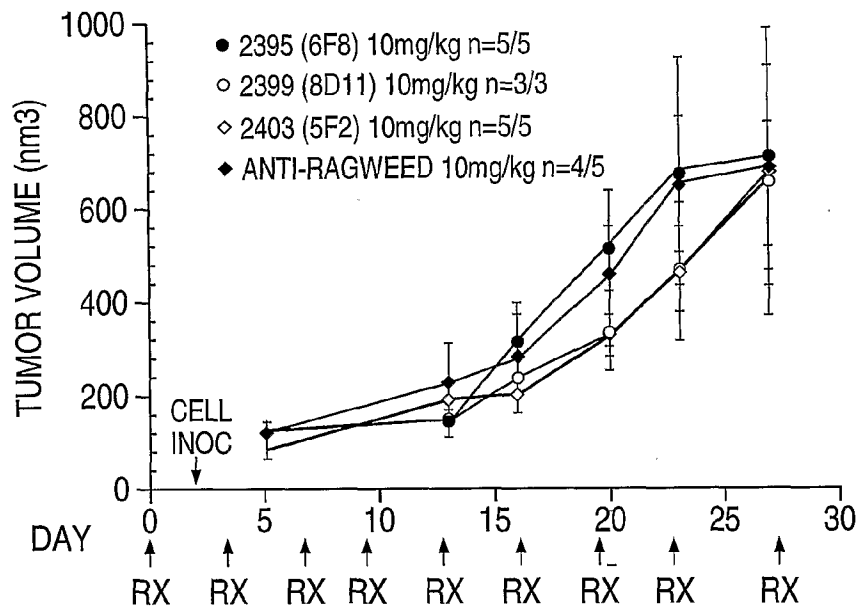


FIG. 18

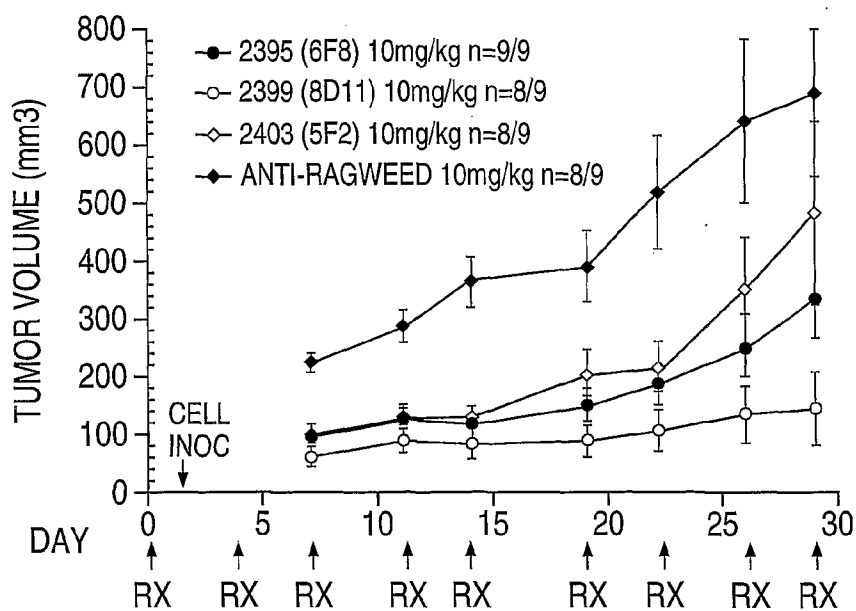


FIG. 19

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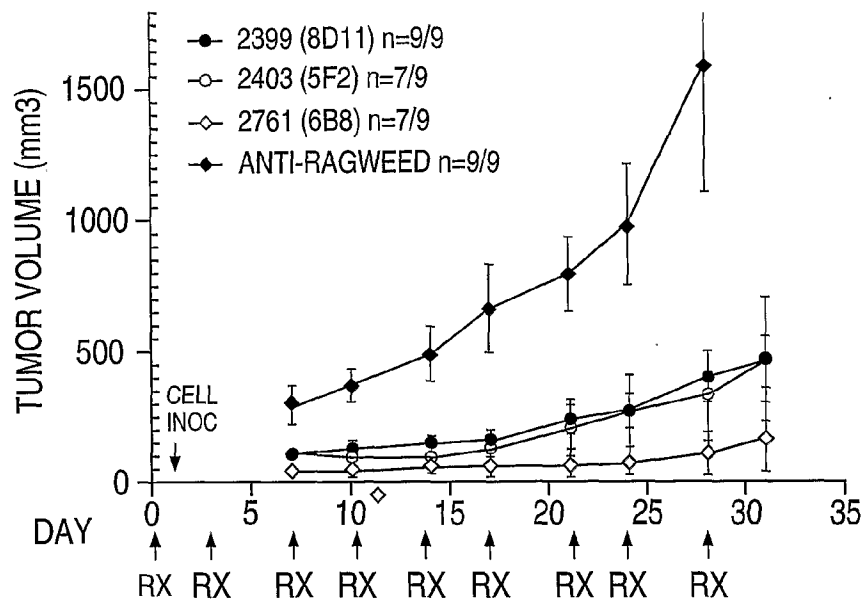


FIG. 20

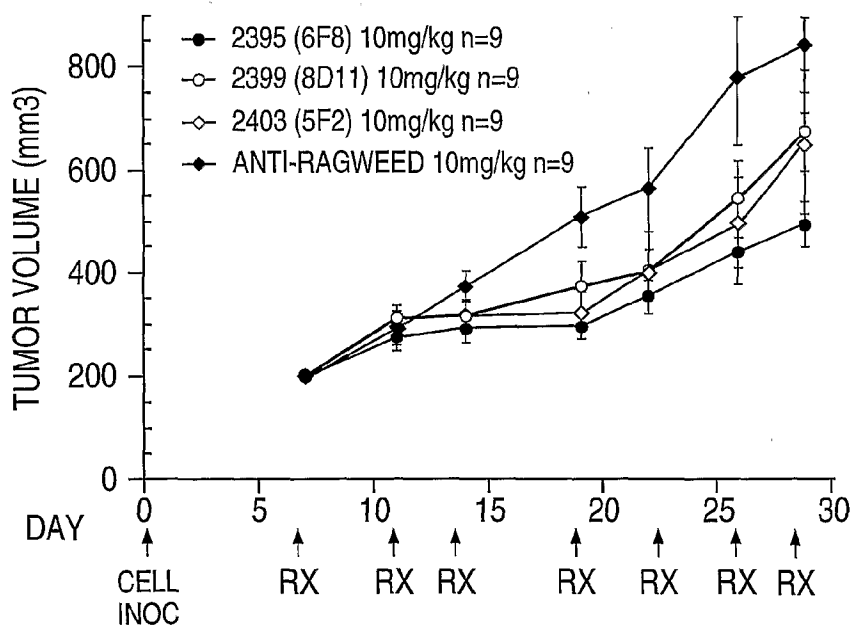
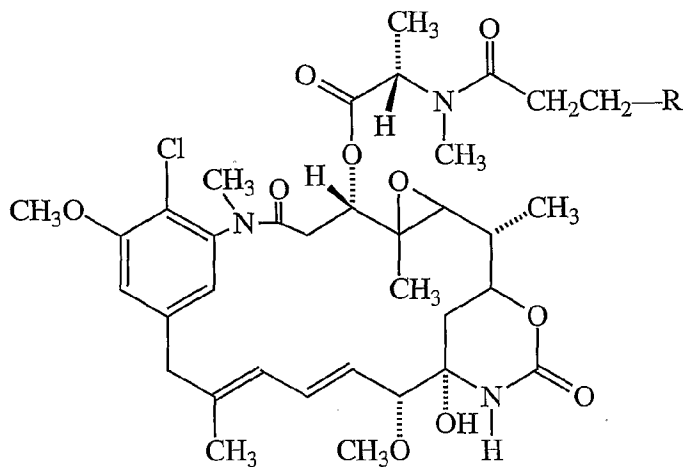
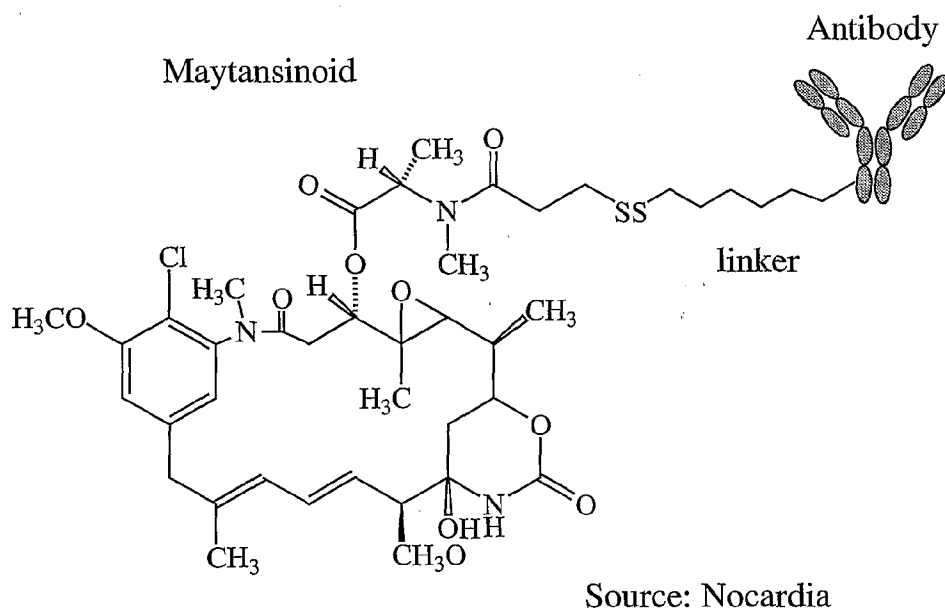
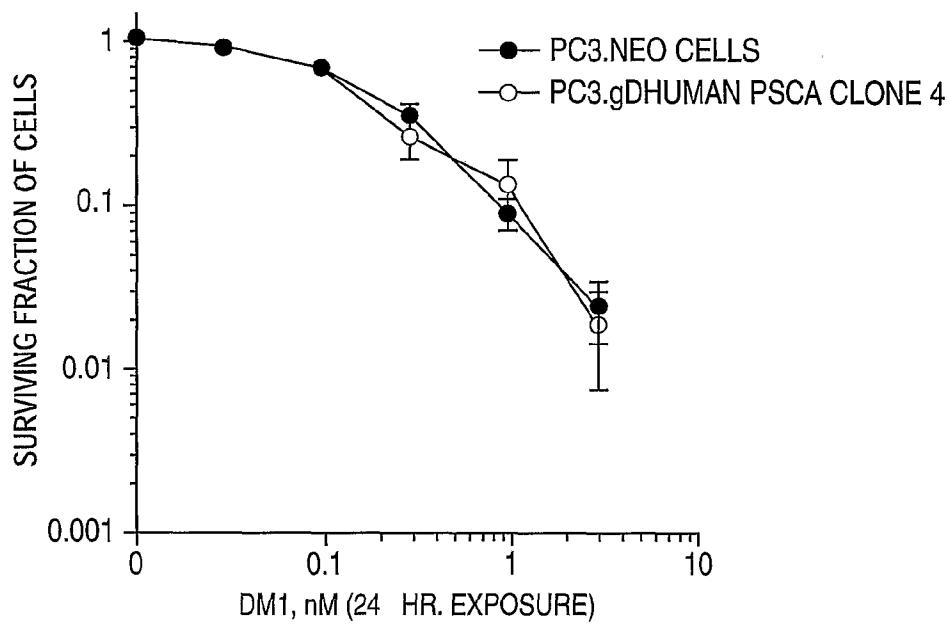
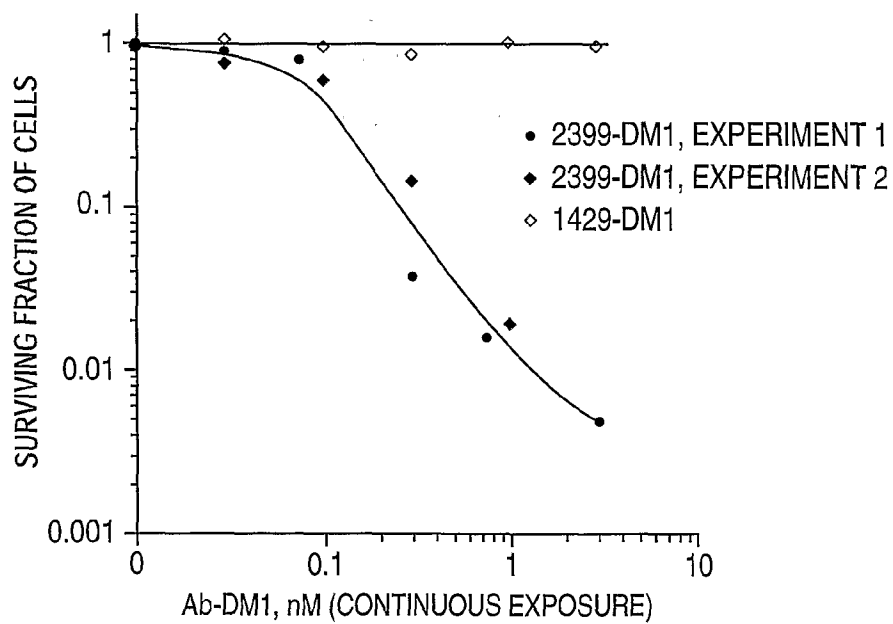


FIG. 21

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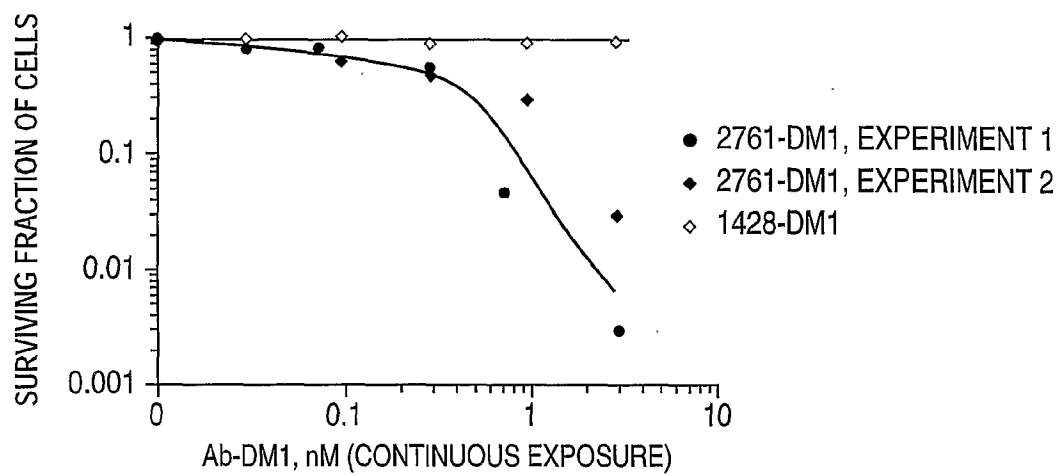
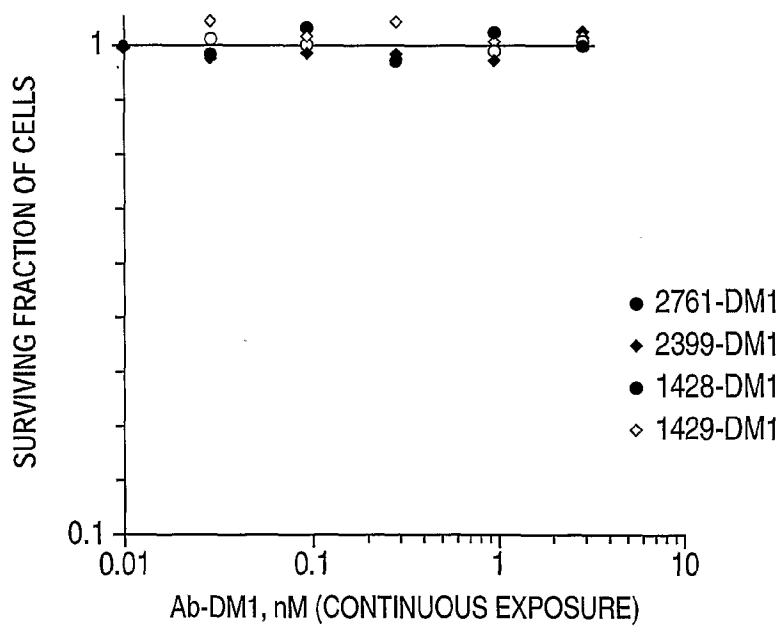
**FIG. 22****FIG. 23**

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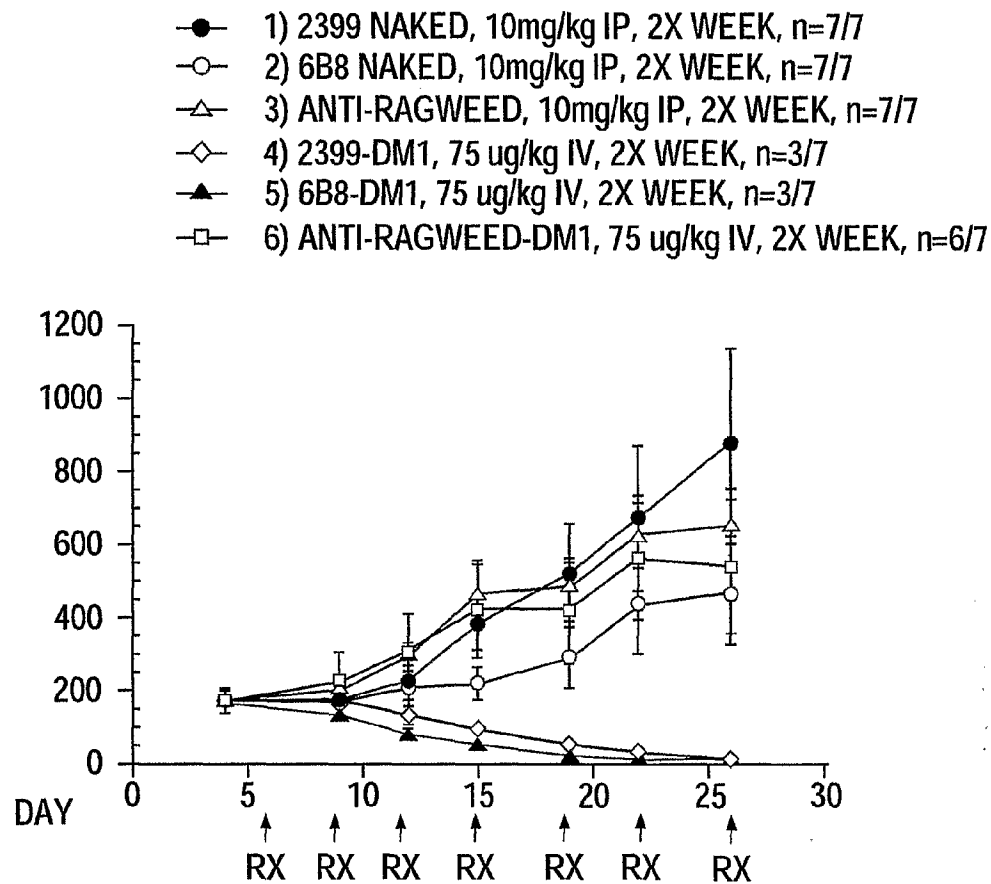
**FIG. 24****FIG. 25**



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**FIG. 26****FIG. 27**

18/18

**FIG. 28**

## Sequence Listing

<110> Genentech, Inc.  
 Devaux, Brigitte  
 Keller, Gilbert-Andre.  
 Koeppen, Hartmut  
 Lasky, Laurence A.

<120> Anti-Tumor Antibody Compositions and Methods of Use

<130> P1777R1PCT

<141> 2000-10-27

<150> US 60/162,558  
 <151> 1999-10-29

<150> US 60/182,872  
 <151> 2000-02-16

<160> 25

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 <213> Homo sapiens

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 1 5 10 15  
 Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser Cys Lys Ala Gln Val  
 20 25 30  
 Ser Asn Glu Asp Cys Leu Gln Val Glu Asn Cys Thr Gln Leu Gly  
 35 40 45  
 Glu Gln Cys Trp Thr Ala Arg Ile Arg Ala Val Gly Leu Leu Thr  
 50 55 60  
 Val Ile Ser Lys Gly Cys Ser Leu Asn Cys Val Asp Asp Ser Gln  
 65 70 75  
 Asp Tyr Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp  
 80 85 90  
 Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala Ala Ala  
 95 100 105  
 Ile Leu Ala Leu Leu Pro Ala Leu Gly Leu Leu Leu Trp Gly Pro  
 110 115 120  
 Gly Gln Leu

<210> 2  
 <211> 372  
 <212> DNA  
 <213> Homo sapiens

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 Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly  
 1 5 10

ttg gcc ctg cag cca ggc act gcc ctg ctg tgc tac tcc 75  
 Leu Ala Leu Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser  
           15                          20                          25

tgc aaa gcc cag gtg agc aac gag gac tgc ctg cag gtg 114  
 Cys Lys Ala Gln Val Ser Asn Glu Asp Cys Leu Gln Val  
                           30                          35

gag aac tgc acc cag ctg ggg gag cag tgc tgg acc gcg 153  
 Glu Asn Cys Thr Gln Leu Gly Glu Gln Cys Trp Thr Ala  
           40                          45                          50

cgc atc cgc gca gtt ggc ctc ctg acc gtc atc agc aaa 192  
 Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile Ser Lys  
                           55                          60

ggc tgc agc ttg aac tgc gtg gat gac tca cag gac tac 231  
 Gly Cys Ser Leu Asn Cys Val Asp Asp Ser Gln Asp Tyr  
           65                          70                          75

tac gtg ggc aag aag aac atc acg tgc tgt gac acc gac 270  
 Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp  
                           80                          85                          90

ttg tgc aac gcc agc ggg gcc cat gcc ctg cag ccg gct 309  
 Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala  
                           95                          100

gcc gcc atc ctt gcg ctg ctc cct gca ctc ggc ctg ctg 348  
 Ala Ala Ile Leu Ala Leu Leu Pro Ala Leu Gly Leu Leu  
           105                          110                          115

ctc tgg gga ccc ggc cag cta tag 372  
 Leu Trp Gly Pro Gly Gln Leu Xaa  
                           120                          124

<210> 3

<211> 96

<212> PRT

<213> Mus musculus

<400> 3

Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser  
       1                          5                          10                          15

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln  
                           20                          25                          30

Ser Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly  
                           35                          40                          45

Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr  
                           50                          55                          60

Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr  
                           65                          70                          75

Cys Met Gln His Leu Glu Ser Pro Phe Thr Phe Gly Ser Gly Thr  
                           80                          85                          90

Lys Leu Glu Ile Lys Arg  
                           95

<210> 4

<211> 115  
 <212> PRT  
 <213> Mus Musculus

<220>  
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 <222> 46-48, 50-52  
 <223> unknown amino acid

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 Ser Gly Tyr Thr Phe Thr Asn Tyr Trp Met Asn Trp Val Lys Gln  
                     20                    25                    30  
 Arg Pro Gly Arg Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ser  
                     35                    40                    45  
 Xaa Xaa Xaa Thr Xaa Xaa Xaa Gln Thr Phe Lys Asp Lys Ala Thr  
                     50                    55                    60  
 Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Ile Gln Leu Ser  
                     65                    70                    75  
 Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Ile Thr  
                     80                    85                    90  
 Ala Ala Ile Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr  
                     95                    100                    105  
 Val Ser Ser Ala Lys Thr Thr Gly Pro Ser  
                     110                    115

<210> 5  
 <211> 113  
 <212> PRT  
 <213> Mus musculus

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 Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro  
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 Gly Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu  
                     20                    25                    30  
 His Ser Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro  
                     35                    40                    45  
 Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala  
                     50                    55                    60  
 Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala  
                     65                    70                    75  
 Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val  
                     80                    85                    90  
 Tyr Tyr Cys Leu Gln His Leu Glu Tyr Pro Tyr Thr Phe Gly Gly  
                     95                    100                    105  
 Gly Thr Lys Leu Glu Leu Lys Arg  
                     110

<210> 6  
 <211> 124  
 <212> PRT  
 <213> Mus musculus

<400> 6  
 Gln Val Gln Val Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly  
     1                    5                    10                    15  
 Ala Pro Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr  
                     20                    25                    30  
 Asn Tyr Trp Leu Asn Trp Val Lys Gln Arg Pro Gly Arg Gly Leu  
                     35                    40                    45  
 Glu Trp Ile Gly Arg Ile Asp Pro Ser Asp Ser Glu Ile His Tyr  
                     50                    55                    60  
 Asp Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser  
                     65                    70                    75  
 Ser Ser Thr Ala Tyr Ile Gln Leu Ser Ser Leu Thr Ser Glu Asp  
                     80                    85                    90  
 Ser Ala Val Tyr Tyr Cys Ala Leu Thr Gly Ile Tyr Ala Met Ala  
                     95                    100                    105  
 Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Lys Thr  
                     110                    115                    120  
 Thr Gly Pro Ser

<210> 7  
 <211> 113  
 <212> PRT  
 <213> Mus musculus

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 Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val Thr Ile  
     1                    5                    10                    15  
 Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu  
                     20                    25                    30  
 Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro  
                     35                    40                    45  
 Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Thr Leu Asp  
                     50                    55                    60  
 Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp  
                     65                    70                    75  
 Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val  
                     80                    85                    90  
 Tyr Tyr Cys Trp Gln Gly Thr His Phe Pro Arg Thr Phe Gly Gly  
                     95                    100                    105  
 Gly Thr Lys Leu Glu Ile Lys Arg  
                     110

<210> 8

<211> 121  
 <212> PRT  
 <213> Mus musculus

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 Glu Val Gln Leu Gln Gln Ser Gly Pro Asp Leu Glu Lys Pro Gly  
     1                    5                    10                    15  
 Ala Ser Val Lys Ile Ser Cys Lys Pro Ser Gly Asn Ser Phe Thr  
                     20                    25                    30  
 Gly Tyr Tyr Ile His Trp Val Lys Gln Ser His Gly Lys Ser Leu  
                     35                    40                    45  
 Glu Trp Ile Gly Arg Val Asp Pro Asn Asn Gly Phe Thr Ser Tyr  
                     50                    55                    60  
 Asn Gln Lys Phe Lys Gly Lys Ala Ile Leu Thr Val Asp Lys Ser  
                     65                    70                    75  
 Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp  
                     80                    85                    90  
 Ser Ala Val Tyr Tyr Cys Val Gly Asn Phe Phe Asp Ser Trp Gly  
                     95                    100                    105  
 Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Gly Pro  
                     110                    115                    120  
 Ser

<210> 9  
 <211> 118  
 <212> PRT  
 <213> Mus musculus

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 Pro Gly Ala Glu Leu Val Lys Pro Gly Ala Pro Val Lys Leu Ser  
     1                    5                    10                    15  
 Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Trp Met Asn Trp  
                     20                    25                    30  
 Val Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile Gly Arg Ile  
                     35                    40                    45  
 Asp Pro Ser Asp Ser Glu Thr Gln Tyr Asn Gln Thr Phe Lys Asp  
                     50                    55                    60  
 Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Ile  
                     65                    70                    75  
 Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
                     80                    85                    90  
 Ala Ile Thr Ala Ala Ile Ala Met Asp Tyr Trp Gly Gln Gly Thr  
                     95                    100                    105  
 Ser Val Thr Val Ser Ser Ala Lys Thr Thr Gly Pro Ser  
                     110                    115

<210> 10  
 <211> 238

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; sequence is chimeric mouse/human

&lt;400&gt; 10

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
  1           5           10           15

Gly Val His Ser Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val
          20           25           30

Pro Val Thr Pro Gly Glu Ser Val Ser Ile Ser Cys Arg Ser Ser
          35           40           45

Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr Trp Phe
          50           55           60

Leu Gln Arg Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Met
          65           70           75

Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
          80           85           90

Ser Gly Thr Ala Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu
          95          100          105

Asp Val Gly Val Tyr Tyr Cys Leu Gln His Leu Glu Tyr Pro Tyr
          110          115          120

Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala
          125          130          135

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
          140          145          150

Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
          155          160          165

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
          170          175          180

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
          185          190          195

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
          200          205          210

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu
          215          220          225

Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
          230          235

```

&lt;210&gt; 11

&lt;211&gt; 466

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; sequence is chimeric mouse/human

&lt;400&gt; 11



Met	Gly	Trp	Ser	Cys	Ile	Ile	Leu	Phe	Leu	Val	Ala	Thr	Ala	Thr		1	5	10	15
Gly	Val	His	Ser	Gln	Val	Gln	Val	Gln	Gln	Pro	Gly	Ala	Glu	Leu		20	25	30	
Val	Lys	Pro	Gly	Ala	Pro	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Gly		35	40	45	
Tyr	Thr	Phe	Thr	Asn	Tyr	Trp	Leu	Asn	Trp	Val	Lys	Gln	Arg	Pro		50	55	60	
Gly	Arg	Gly	Leu	Glu	Trp	Ile	Gly	Arg	Ile	Asp	Pro	Ser	Asp	Ser		65	70	75	
Glu	Ile	His	Tyr	Asp	Gln	Lys	Phe	Lys	Asp	Lys	Ala	Thr	Leu	Thr		80	85	90	
Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Ile	Gln	Leu	Ser	Ser	Leu		95	100	105	
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Leu	Thr	Gly	Ile		110	115	120	
Tyr	Ala	Met	Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser		125	130	135	
Ser	Ala	Lys	Thr	Thr	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser		140	145	150	
Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val		155	160	165	
Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly		170	175	180	
Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser		185	190	195	
Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser		200	205	210	
Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro		215	220	225	
Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp		230	235	240	
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly		245	250	255	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu		260	265	270	
Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val		275	280	285	
Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly		290	295	300	
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr		305	310	315	
Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln					

				320					325					330
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys
				335					340					345
Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly
				350					355					360
Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu
				365					370					375
Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly
				380					385					390
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln
				395					400					405
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp
				410					415					420
Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg
				425					430					435
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
				440					445					450
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly
				455					460					465

Lys

&lt;210&gt; 12

&lt;211&gt; 218

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; sequence is chimeric mouse/human

&lt;400&gt; 12

Asp	Val	Val	Met	Thr	Gln	Thr	Pro	Leu	Thr	Leu	Ser	Val	Thr	Ile
1				5					10					15
Gly	Gln	Pro	Ala	Ser	Ile	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Leu
				20					25					30
Asp	Ser	Asp	Gly	Lys	Thr	Tyr	Leu	Asn	Trp	Leu	Leu	Gln	Arg	Pro
				35					40					45
Gly	Gln	Ser	Pro	Lys	Arg	Leu	Ile	Tyr	Leu	Val	Ser	Thr	Leu	Asp
				50					55					60
Ser	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Thr	Asp
				65					70					75
Phe	Thr	Leu	Lys	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val
				80					85					90
Tyr	Tyr	Cys	Trp	Gln	Gly	Thr	His	Phe	Pro	Arg	Thr	Phe	Gly	Gly
				95					100					105
Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val
				110					115					120

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala  
 125 130 135  
 Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 140 145 150  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
 155 160 165  
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu  
 170 175 180  
 Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys  
 185 190 195  
 Val Tyr Ala Cys Glu Thr His Gln Gly Leu Ser Ser Pro Val Thr  
 200 205 210  
 Lys Ser Phe Asn Arg Gly Glu Cys  
 215

&lt;210&gt; 13

&lt;211&gt; 222

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; sequence is chimeric mouse/human

&lt;400&gt; 13

Glu Val Gln Leu Gln Gln Ser Gly Pro Asp Leu Glu Lys Pro Gly  
 1 5 10 15  
 Ala Ser Val Lys Ile Ser Cys Lys Pro Ser Gly Asn Ser Phe Thr  
 20 25 30  
 Gly Tyr Tyr Ile His Trp Val Lys Gln Ser His Gly Lys Ser Leu  
 35 40 45  
 Glu Trp Ile Gly Arg Val Asp Pro Asn Asn Gly Phe Thr Ser Tyr  
 50 55 60  
 Asn Gln Lys Phe Lys Gly Lys Ala Ile Leu Thr Val Asp Lys Ser  
 65 70 75  
 Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp  
 80 85 90  
 Ser Ala Val Tyr Tyr Cys Val Gly Asn Phe Phe Asp Ser Trp Gly  
 95 100 105  
 Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Gly Pro  
 110 115 120  
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly  
 125 130 135  
 Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 140 145 150  
 Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His  
 155 160 165  
 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser

```

170                               175                               180
Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr
185                               190                               195

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
200                               205                               210

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
215                               220

<210> 14
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence is a primer

<400> 14
aaggctgtgc tgcttgccct 20

<210> 15
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence is a primer

<400> 15
gagtggcaca aaggcctggg 20

<210> 16
<211> 372
<212> DNA
<213> Macaca fascicularis

<400> 16
atg aag gct gtg ctg ctt gcc ctg ttg atg gca ggc 36
Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly
1 5 10

ttg gcc ctg cag cca ggc act gcc ctg ctg tgc tac tcc 75
Leu Ala Leu Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser
15 20 25

tgc aag gcc cag gtg agc aac gag gac tgc ctg aat gtg 114
Cys Lys Ala Gln Val Ser Asn Glu Asp Cys Leu Asn Val
30 35

gag aac tgc acg cag ccg gag gag cag tgc tgg acc gag 153
Glu Asn Cys Thr Gln Pro Glu Glu Gln Cys Trp Thr Glu
40 45 50

cgc atc cgc gcc gtg ggc ctc ctg acc gtc atc agc aaa 192
Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile Ser Lys
55 60

ggc tgc agc tca aac tgc gtg gat gac tca cag gac tac 231
Gly Cys Ser Ser Asn Cys Val Asp Asp Ser Gln Asp Tyr
65 70 75

tac gtg ggc aag aag aac atc acc tgc tgt gac acc gac 270

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Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp  
           80  85  90  
  
 ttg tgc aac gcc agc ggg gcc cat gca ctg cag ccg gct 309  
 Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala  
                                   95  100  
  
 gct gcc atc ctg gca ctg ctc cct gca ctc agt ctg ctg 348  
 Ala Ala Ile Leu Ala Leu Leu Pro Ala Leu Ser Leu Leu  
           105  110  115  
  
 ctt tgg agc ccc aga cag ctg t ag 372  
 Leu Trp Ser Pro Arg Gln Leu  
                   120                                  123

<210> 17  
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 <212> PRT  
 <213> Macaca fascicularis

<400> 17  
 Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly Leu Ala Leu  
   1                                  5  10  15  
  
 Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser Cys Lys Ala Gln Val  
                                   20  25  30  
  
 Ser Asn Glu Asp Cys Leu Asn Val Glu Asn Cys Thr Gln Pro Glu  
                                   35  40  45  
  
 Glu Gln Cys Trp Thr Glu Arg Ile Arg Ala Val Gly Leu Leu Thr  
                                   50  55  60  
  
 Val Ile Ser Lys Gly Cys Ser Ser Asn Cys Val Asp Asp Ser Gln  
                                   65  70  75  
  
 Asp Tyr Tyr Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp  
                                   80  85  90  
  
 Leu Cys Asn Ala Ser Gly Ala His Ala Leu Gln Pro Ala Ala Ala  
                                   95  100  105  
  
 Ile Leu Ala Leu Leu Pro Ala Leu Ser Leu Leu Leu Trp Ser Pro  
                                   110  115  120  
  
 Arg Gln Leu

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<400> 18  
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   Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly  
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   ttg gcc ctg cag cca ggc act gcc ctg ttg tgc tac tcc 75  
   Leu Ala Leu Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser  
     15  20  25  
  
   tgc aag gcc cag gtg agc aac gag gac tgc ctg aat gtg 114  
   Cys Lys Ala Gln Val Ser Asn Glu Asp Cys Leu Asn Val

30														35													
gag	aac	tgc	acg	cag	ccg	gag	gag	cag	tgc	tg	acc	gag	153														
Glu	Asn	Cys	Thr	Gln	Pro	Glu	Glu	Gln	Cys	Trp	Thr	Glu															
40								45				50															
cgc	atc	cgc	gcc	gtg	ggc	ctc	ctg	acc	gtc	atc	agc	aaa	192														
Arg	Ile	Arg	Ala	Val	Gly	Leu	Leu	Thr	Val	Ile	Ser	Lys															
55								60																			
ggc	tgc	agc	tca	aac	tgc	gtg	gat	gac	tca	cag	gac	tac	231														
Gly	Cys	Ser	Ser	Asn	Cys	Val	Asp	Asp	Ser	Gln	Asp	Tyr															
65				70				75																			
tac	gtg	ggc	aag	aag	aac	atc	acc	tgc	tgt	gac	acc	gac	270														
Tyr	Val	Gly	Lys	Lys	Asn	Ile	Thr	Cys	Cys	Asp	Thr	Asp															
80								85				90															
ttg	tgc	aac	gcc	agc	ggg	gcc	cat	gcc	ctg	cag	cca	gct	309														
Leu	Cys	Asn	Ala	Ser	Gly	Ala	His	Ala	Leu	Gln	Pro	Ala															
95								100																			
gct	gcc	atc	ctg	gca	ctg	ctc	cct	gca	ctc	agc	ctg	ctg	348														
Ala	Ala	Ile	Leu	Ala	Leu	Leu	Pro	Ala	Leu	Ser	Leu	Leu															
105				110								115															
ctt	tg	ggc	ccc	aga	cag	ctg	t	ag	372																		
Leu	Trp	Gly	Pro	Arg	Gln	Leu																					
120				123																							
<210> 19																											
<211> 123																											
<212> PRT																											
<213> Macaca fascicularis																											
<400> 19																											
Met	Lys	Ala	Val	Leu	Leu	Ala	Leu	Leu	Met	Ala	Gly	Leu	Ala	Leu													
1					5					10					15												
Gln	Pro	Gly	Thr	Ala	Leu	Leu	Cys	Tyr	Ser	Cys	Lys	Ala	Gln	Val													
				20					25					30													
Ser	Asn	Glu	Asp	Cys	Leu	Asn	Val	Glu	Asn	Cys	Thr	Gln	Pro	Glu													
				35					40					45													
Glu	Gln	Cys	Trp	Thr	Glu	Arg	Ile	Arg	Ala	Val	Gly	Leu	Leu	Thr													
				50					55					60													
Val	Ile	Ser	Lys	Gly	Cys	Ser	Ser	Asn	Cys	Val	Asp	Asp	Ser	Gln													
				65					70					75													
Asp	Tyr	Tyr	Val	Gly	Lys	Lys	Asn	Ile	Thr	Cys	Cys	Asp	Thr	Asp													
				80					85					90													
Leu	Cys	Asn	Ala	Ser	Gly	Ala	His	Ala	Leu	Gln	Pro	Ala	Ala	Ala													
				95					100					105													
Ile	Leu	Ala	Leu	Leu	Pro	Ala	Leu	Ser	Leu	Leu	Leu	Trp	Gly	Pro													
				110					115					120													
Arg Gln Leu																											

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<210> 25  
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<212> DNA  
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<220>  
<223> Sequence is a PCR primer

<400> 25  
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>73</u> , line <u>8</u> - <u>38-41</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, VA 20110-2209</b>	
Date of deposit <b>24 October 2000</b>	Accession Number <b>PTA-2620, PTA-2621, PTA-2622, PTA-2623</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
NOT APPLICABLE	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g. "Accession Number of Deposit")	

For receiving Office use only	
<input type="checkbox"/> This sheet was received with the international application	
Authorized officer	

For International Bureau use only	
<input checked="" type="checkbox"/> This sheet was received by the International Bureau on <b>29.11.2000</b>	
Authorized officer <b>G. Bahr</b>	



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/29603

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/30 C12N5/10 C12N5/16 C12N15/13 C12N15/67  
 A61K39/395 A61K47/48 A61K51/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, BIOSIS, CHEM ABS Data, EPO-Internal, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 40403 A (UNIV CALIFORNIA) 17 September 1998 (1998-09-17) cited in the application  page 10, line 1 -page 14, line 17 page 23, line 18 -page 25, line 14 page 35, line 5 -page 37, line 35 ---	15,20, 21, 29-32, 46-55
A	REITER R E ET AL: "Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 FEB 17) 95 (4) 1735-40. , XP002089381 the whole document --- -/--	1-56

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

\* Special categories of cited documents :

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*Z\* document member of the same patent family

Date of the actual completion of the international search

22 May 2001

Date of mailing of the international search report

07.06.01

Name and mailing address of the ISA

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Authorized officer

Mennessier, T

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/29603

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 01 05427 A (UNIV CALIFORNIA ;UROGENESYS (US)) 25 January 2001 (2001-01-25) page 24, line 5 -page 35, line 25 page 51, line 17 -page 63, line 7 page 115, line 1 -page 124, line 10 page 139, line 1 -page 141, line 5 ---	1-56
T	SAFFRAN, DOUGLAS C. ET AL: "Anti- PSCA mAbs inhibit tumor growth and metastasis formation an prolong the survival of mice bearing human prostate cancer xenografts." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (FEBRUARY 27, 2001) VOL. 98, NO. 5, PP. 2658-2663. PRINT. , XP000999115 the whole document ---	15,20, 21, 29-32, 46-55
P,X	AMARA, NORDINE (1) ET AL: "Monoclonal antibodies against prostate stem cell antigen inhibit prostate cancer tumour formation i SCID mice." EUROPEAN UROLOGY, (MARCH, 2000) VOL. 37, NO. SUPPL. 2, PP. 105, ABSTRACT 420. PRINT. MEETING INFO.: XVTH CONGRESS OF THE EUROPEAN ASSOCIATION OF UROLOGY BRUSSELS, BELGIUM APRIL 12-15, 2000 , XP000999929 the whole document ---	15,20, 21, 29-32, 46-55
P,X	WO 00 32752 A (UNIV CALIFORNIA) 8 June 2000 (2000-06-08) page 17, line 23 -page 27, line 2 page 42, line 1 -page 46, line 11 -----	1-56

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/29603

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 34-45, insofar as the cancer cells to be treated have not been isolated from the body, and claims 46-55, as a whole, are directed to a method of treatment of the human/animal body by therapy, the search has been carried out and based on the alleged effects of the antibody.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/29603

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9840403 A	17-09-1998	AU 6548198 A EP 0981541 A	29-09-1998 01-03-2000
WO 0105427 A	25-01-2001	AU 6364100 A	05-02-2001
WO 0032752 A	08-06-2000	AU 2166800 A	19-06-2000